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Conseil Permanent International pour l'Exploration de la Mer

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RAPPORTS ET PROCÈS-VERBAUX DES RÉUNIONS

Volume 144

CONTRIBUTIONS TO PLANKTON SYMPOSIUM 1957

Measurements of Primary Production in the Sea

En Commission chez

ANDR. FRED. HØST & FILS

COPENHAGUE

Prix: Kr. 30.00

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Editorial Notice

The contributions by P. S. Davis and H. J. Jitts entitled: "A method for the determination of chlorophyll in sea water" and "The ^{14}C method for measuring CO_2 uptake in marine productivity studies", respectively, have appeared as Reports Nos. 7 and 8 of the Marine Biological Laboratory, Cronulla and have therefore been omitted in the present volume.

The papers by Brouardel & Rinck and Kriss, read at the Symposium will appear in the series *Journal du Conseil.*

Introduction

THE symposium on "Measurements of Primary Production in the Sea" held at Bergen 27.—29. September 1957 was favoured by great interest from the specialists working in the field. Thirty-one contributions were presented at the meeting; 75 participants were registered. The contributions were—with some few exceptions—distributed to the participants a month before the time of the symposium. During the symposium, therefore, the papers were presented in the form of short reviews and most of the time was used for discussions. This turned out to be a very fortunate situation. Generally there was sufficient time for the discussions.

The discussions were not taken down in shorthand. The reviewer Dr. CUSHING, however, made notes on all the main points. The participants of the symposium recommended that the discussions should be printed *in extenso*, but this was precluded on grounds of economy. A short review will be given instead, based on the notes made by Dr. CUSHING.

Five sessions were held each with its special topic. The last session, however, was mainly used for the final approval of recommendations from the *ad hoc* working committees which were appointed during the meeting. Four of these committees presented their recommendations which were adopted unanimously by the participants. These sets of recommendations are printed below. The fifth working committee dealing with the consumption of nutrient salts as a means of measuring production did not find it apposite to present any recommendation.

During the discussions it was found useful to present definitions of the terms generally used. The same committee dealing with these terms made recommendation of equivalents being useful for phytoplankton work. It was pointed out that these equivalents are to be used with great care.

As the most essential points discussed during the two first sessions (about the methods) are presented in the recommendations from the *ad hoc* committees, the reviews of the discussions from these sessions can be reported briefly.

Session on methods of estimating standing crop in phytoplankton

The papers and the discussions clearly showed the difficulties in estimating the standing crop. From all quarters present it was stressed that net methods should not be employed in quantitative phytoplankton studies.

It was agreed that adequate techniques for counting phytoplankton are available. On the other hand, absolutely satisfactory preservatives for all phytoplankton organisms do not seem to be accessible. The preservation of the ultraplankton organisms seems to be especially difficult.

The use of chlorophyll for measuring either the standing crop of phytoplankton or the photosynthetic potential—i. e., the rate of light-saturated photosynthesis—was much discussed. The opinions were rather divergent. In addition to point I (a) 3 in the report of the Committee on Chlorophyll, it may perhaps be quoted that under uniform conditions chlorophyll or total pigments seem to be adequate measures both of the stock of algae and of the photosynthetic potential, but on comparing one kind of water with another, or one depth with another, or one season with another any definite relation both to the stock and to the potential may be somewhat questionable.

The discussion on chlorophyll showed both the great interest from the participants in measuring this pigment, and the great need for intensifying the development of standard techniques and establishing a sound basis for using the measurements.

During the session the estimation of carbon in phytoplankton was discussed. Finally it was stressed by several participants that we must not forget to state the composition of the plankton when estimating the standing crop of phytoplankton. As Dr. KETCHUM said: "We must not forget that there are different species each of which may react differently to definite environmental conditions. Now much laboratory work on culture has been done on the tough weeds of the sea, and so some of our generalizations may be too simple."

Session on methods of measurements of production

The rather pessimistic view held by the first speaker, Dr. COOPER, regarding the use of the consumption of nutrient salts as a means of measuring production was commented upon by Mr. STEELE. He had chosen an area hydrographically simple where it was probable that vertical mixing was the controlling factor. Thus some of COOPER's difficulties were by-passed.

In the discussion on experimental methods it was stressed that we are unable to imitate absolutely the natural conditions for the plankton algae. However, we must try to make the experimental conditions as close to the natural as possible. Many difficulties may turn up, some of which we may overcome. For example, Dr. RINCK presented a special *in situ* technique using carbon-14, in which the tracer was added to the experimental water without first bringing the water sample to the surface. According to Dr. RINCK's opinion a higher photosynthetic rate may be measured in this way in subsurface samples. The importance of stirring in the experimental vessels was shown experimentally by Professor GESSNER and Dr. DOTY. Dr. RODHE had noticed that cells only sink a little in an irregularly shaped bottle, but that they do so to a much greater extent in a spherical one, in which therefore a slower rate of photosynthesis may take place.

The late-afternoon depression of the photosynthetic capacity was thoroughly discussed. There seems to be more than one cause for the depression. Light inhibition perhaps seems to be the

most important one. Changes in pH and the establishment of crusts on the surfaces of the algae may be other causes of diurnal variation in extremely productive lakes such as shown by Dr. Ohle. The turbulence during the night under natural conditions will give rise to a washing effect. If water samples are enclosed in bottles such washing does not take place. This may cause a considerable decrease of the rates of photosynthesis and respiration.

The Convener stressed the different difficulties in using the oxygen technique and the carbon-14 technique. This raised only a discussion on fitting a self-absorption curve, the stage of the carbon-14 technique which perhaps gives rise to most difficulties.

Dr. Fogg presented his paper on extracellular products of phytoplankton. Although his experiments did not indicate any serious influence of extracellular products on the estimations of phytoplankton when using the carbon-14 technique it was agreed that further investigations would be useful. Professor Fedosov's paper was first presented before session 4, together with a paper by Professor Kriss (to be published in *Journal du Conseil*), according to which a new type of autotrophic organism was found in the Black Sea, namely a radio-autotrophic organism. This raised some opposition during the discussion. The papers by Dr. Bogorov and Dr. Winberg were not presented during the symposium. Together with Professor Fedosov's paper they describe the activities in Russia concerning the investigation of the primary productivity in the sea.

Session on measurements of primary production

The papers presented during this session showed that at present primary production is measured in many regions of the sea all over the world. The carbon-14 technique was used by most workers, but different modifications had been employed: (a) *in situ* measurements, (b) simulated *in situ* measurements with samples exposed to natural illumination adjusted to that of natural conditions by means of neutral filters and, (c) incubator measurements with samples exposed to artificial illumination of constant intensity. It was agreed that *in situ* measurements were preferable but not always possible.

It was a great benefit to the marine workers that limnologists took part in the symposium. Dr. Rodhe's paper dealing with the primary production in lakes gave rise to several interesting discussions. In some experimental series in Lake Erken the primary production was measured either in a single experiment during the whole day or by summing up the results of short time experiments covering the whole day. These experiments will turn out to be of great importance when discussing the late-afternoon depression in production also found in the sea.

The many papers presented during the session gave a clear evidence that the production pattern varies much from one area to another even if only the temperate northern waters are taken into consideration. In some areas we may find a typical spring maximum followed by a summer minimum which again is succeeded by an autumn maximum. The measurements from the offshore water outside New York, presented by Dr. Ketchum during the last session, showed a typical example of such conditions.

The measurements from the Great Belt, presented by the Convener, showed in contrast a situation where the maximum of production is found during the high summer. The conditions in other areas again is different both from the offshore area outside New York and the Great Belt.

It is quite unneccessary to go into details of all the single papers presented. They all give important information about the different regions investigated. Mr. BERGE's paper, however, deserves to be especially mentioned. During a single summer month a close net of productivity stations was worked in the Norwegian Sea. It was shown that the rate of production both per volume at a definite light intensity or per surface unit at standard light conditions may be treated just as chemical or physical properties like salinity and temperature. Investigations in smaller scale by other workers have given the same picture. For all future work dealing with productivity measurements, BERGE's statement will be of importance. A single measurement even in a hydrographically complicated area like the Norwegian Sea does not present a random value but is a property characteristic of the water mass in question.

At the first glance Mr. BERGE's statement seems to contradict RODHE's measurements from Lake Erken, where at a fixed station considerable variations were found in the rate of production from day to day, primarily due to currents, according to RODHE. It must, however, be mentioned that the rate of production in a lake like Erken may vary considerably from one part of the lake to another. If a special water mass had been followed, the daily variation inside this water mass very likely may not have varied considerably.

Session on environmental conditions for primary production

Only Dr. KETCHUM's paper: "Productivity in relation to nutrients" gave rise to an exhaustive discussion. All taking part in the discussion agreed that the replenishment of nutrient is the factor which in most cases determines the fertility of an aquatic environmental. But there was disagreement about the way in which lack in nutrients limits productivity. This point is at present perhaps the most important one for the exploration of the background of marine productivity. The discussion must be presented in somewhat more detail.

According to Dr. KETCHUM, as presented in his paper, lack of nutrients produces deficient plankton algae. These consequently should have a low net: gross photosynthesis ratio even at an optimum light intensity. Against this the Convener pointed out that the low net: gross photosynthesis ratio as presented by KETCHUM may be considered probably as merely artefacts. On recent Danish cruises about 100 experimental series have been made with marine plankton from all sorts of water—both eutrophic and oligotrophic, both tropical, temperate, and arctic—showing rate of photosynthesis as a function of light intensity. By extrapolation a fairly correct measure of the rate of respiration can be obtained—cf. Fig. 2, p. 41 of this publication. A low net: gross photosynthesis ratio has been found according to none of these numerous experimental series.

In contrast to the Woods Hole Group the Convener believes that the standing crop of phytoplankton and its production is rather stable when the hydrographic conditions are stable.

It would otherwise be extremely difficult to explain, for example, HENTSCHEL's results from the "Meteor" expedition in the South Atlantic. He showed that in a special type of water the standing crop of algae remains exceedingly steady in regard to both place and time. If in higher latitudes the conditions for the growth of phytoplankton change, the phytoplankton seems to be able in a relatively short time to change the standing crop into the size and the composition of species best suited for the new conditions.

It must be considered very fortunate that the extremely important question concerning the influence of nutrient salts was so thoroughly discussed. It is also important that the different views were put clearly forward. The problem is vulnerable both to experiments and to field investigations. It should be easy to come to a generally acceptable point of view.

In many other respects the symposium gave an opportunity to make clear where the main technical difficulties and problems are to be found. The investigators dealing with the primary production in the sea certainly have got impulses for their work in the near future. This was the main purpose of the symposium.

E. STEEMANN NIELSEN

D. H. CUSHING

Recommendations on Studies of Phytoplankton Populations

Mr. R. Holmes, Prof. T. Braarud (Chairman), Dr. W. Rodhe,
Mr. J. Grøntved, Dr. J. Lund

I. Methods and their improvement

A. Methods for general studies, not aiming at a recording of the total population.

1. Net methods should not be employed in quantitative phytoplankton studies.
2. Filtration and sedimentation methods are recommended for concentrating the plankton. (Preservative for marine work: neutralized formalin.)
3. Time-saving counting operations should be resorted to whenever possible.
4. Research should be carried out to determine the minimum concentration of neutralized formalin needed for securing safe storage of samples without interference of micro-biological process even at high temperatures.

B. Methods for population studies in connexion with data on primary production.

1. There is an urgent need for a method of preserving sea-water samples whereby naked flagellates and coccolithophorids retain their morphological characteristics.
2. As soon as a satisfactory preservative for these minute forms is available enumeration of phytoplankton populations for this special purpose should be extended to include the ultraplankton fraction so that data are provided for the total photosynthetic population.
3. As a suitable method for this purpose the following is recommended:
   Counting according to the Utermöhl technique of 200, 50, and 2 ml. samples preserved with neutralized formalin and of a fraction of a 1 ml. sample preserved so as to give adequate data on the ultraplankton.
4. When the data on phytoplankton numbers are used as a basis for computing biomass, the method of calculation should be given in detail. It would be most useful if all papers on phytoplankton populations included information on the volumes of the species occurring.

C. Collaboration in work on improving methods.
The attention of the ICES should be directed to the pressing need of special studies on methods of phytoplankton enumeration. It is recommended that the Plankton Committee of the ICES approaches the Committee on Methods of the International Association of Limnology in order to promote joint activities towards improving the quantitative methods in phytoplankton studies.

II. Culture work

Research on isolation and culturing of phytoplankton species should be initiated in order to provide objects for experimental studies on the physiology and ecology of characteristic representatives of the main taxonomic and biogeographical groups.

Report of the Committee on Chlorophyll

Prof. J. Krey (Chairman), Dr. L. R. Blinks, Dr. Bostwick H. Ketchum,
Mr. Ronald I. Currie, Dr. Dayton E. Carritt

I. Relationship between chlorophyll and standing crop

(a) Chlorophyll measurements are essential because:
1. Chlorophyll is a unique component of the plant cell.
2. It is essential for photosynthesis.
3. Under *uniform conditions*, especially when nutrients are not limiting and when the population is actively growing, it is probably a useful index of the size of the standing crop.

(b) Nevertheless, the ratios of chlorophyll to carbon, nitrogen, albumen, phosphorus, or cell volume will vary as a result of variations in:—
1. Light intensity.
2. Nutrient concentrations:—
   (i) iron,
   (ii) nitrogen,
   (iii) phosphorus,
   (iv) probably many others.
3. Physiological state of the organism:—
   (i) exponential growth period,
   (ii) senescent population.
4. Species composition of populations.

II. Relationships between chlorophyll and photosynthesis

(a) Correlations have been reported:—
1. Between total chlorophyll and ^{14}C assimilation.
2. Between total pigments and ^{14}C assimilation.
3. Between chlorophyll *a* and photosynthesis as measured by the oxygen method, occasionally when poor correlation with ^{14}C assimilation was found.

III. Method of determination of chlorophyll

The objective of the investigation will determine the method to be used for the determination of chlorophyll, and so the accuracy and precision obtained. Then general types of studies can be recognized:—

1. For pigment studies *per se*, in which case chromatographic methods of separation of pigment are essential and spectrophotometric and spectrofluorometric methods yield results with the highest precision and accuracy.
2. For the measurement of the concentration of or characterization of plankton pigments in connexion with photosynthesis studies, measurements with filter photometers, preferably such with narrow band filters will give adequate results.
3. For studies in which variables, such as standing stock are to be related to total pigments, without characterization of individual pigment components, visual comparison methods using artificial standards will be adequate.

Standard samples of chlorophyll *a*, for calibration of method and instruments, can be obtained from Sandoz, A.G., Basel. These samples are crystalline chlorophyll *a* of highest chromatographic purity, and should be furnished in sealed ampoules containing known weights of the compound.

Report of the Committee on Methods for the Measurement of Primary Production

Dr. John H. Ryther (Chairman), Prof. Maxwell S. Doty, Prof. E. Steemann Nielsen, Mr. Grim Berge, Dr. G. E. Fogg

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A. Problems recommended for further study by investigators specializing in the measurement of primary production.

1. Light intensity-photosynthesis curves on phytoplankton living under different conditions (seasonal, regional variations from varying depths within the euphotic zone).
2. Diurnal variability of the photosynthetic potential.
3. Influence of weather on primary production.
4. Spatial distribution of phytoplankton (patchiness) and resulting sampling problems.
5. Assessment of sampling and analytical errors in productivity measurements.
6. Loss of assimilated ^{14}C from losses of organic metabolites by phytoplankton living under various environmental conditions.
7. The assimilation number (photosynthesis per unit chlorophyll at optimum light intensity)—seasonal, regional variations, effect of depth within the euphotic layer. The use of chlorophyll as an index of photosynthesis.
8. The effect and desirability of agitation of experimental bottles.

B. Recommendations and suggestions for investigators specializing in related fields (i.e., fisheries research) who wish to initiate productivity studies in conjunction with other investigations.

1. It was agreed that the carbon-14 technique, at the present moment is a most useful technique for measuring production of organic matter in the sea, and that every effort should be made to facilitate workers in the field to utilize the technique.
2. It is suggested that a central agency be established, for example at Charlottenlund under the direction of Prof. E. Steemann Nielsen, which would provide standardized ampoules of ^{14}C, counting of ^{14}C samples, and/or calculation of carbon assimilation rates. This agency could process an amount of 10,000 samples per year at a cost of approximately $ 3,000, (plus the cost of the tracer). It would pay the run of the agency. It is proposed that UNESCO be approached concerning the possibility of their providing the necessary funds for establishing the agency and guaranteeing the economy during the first years.
3. In most smaller laboratories and research groups in the initiation of such studies, field kits for sample collecting and processing, including water samplers, filtration apparatus, filters, etc., will be provided on request and at cost by Prof. E. Steemann Nielsen, Prof. Maxwell S. Doty, and Dr. John H. Ryther.
4. It is suggested that the following sampling and experimental techniques be employed:—

   Samples should be taken from several representative depths within the euphotic layer with a glass, plastic, or other type non-metallic, non-toxic sampler.

   One or more of the following type of ^{14}C assimilation experiments should be made, listed in order of preferability:—

   In situ measurements, with bottles resuspended at the depths from which samples were taken, for periods of sunrise to noon, noon to sunset, or sunrise to sunset.

   Simulated *in situ* measurement with samples exposed to natural illumination in a temperature-controlled container on shipboard and illumination adjusted to that of natural conditions by means of neutral density filters. Such experiments to be of the same duration as *in situ* experiments.

Incubator measurements with samples exposed to artificial illumination of constant intensity and controlled temperature for periods of 4—12 hours.

In view of the evidence of diurnal variability in photosynthetic potential of phytoplankton, it is suggested that sampling times and experimental periods be standardized, as far as possible, at least for a given investigator working in a given area. Where convenient, it is further suggested that both *in situ* and shipboard measurements be started at astronomical noon.

It is recommended that sampling and experimental techniques, other than those described above be those described in the publications of Prof. E. Steemann Nielsen.

5. Investigators are urged to take advantage of such existing facilities as lightships, weather ships, etc. to supplement and extend productivity measurements beyond the limits possible by research vessels alone.

A Central Agency for ^{14}C Determination

UNESCO has now provided funds for establishing a central agency, under the direction of Prof. Steemann Nielsen at the »Danish Institute for Fisheries and Marine Research«, Charlottenlund Slot, Denmark.

At this agency standardized ampoules of ^{14}C may be obtained, the membrane filters with the plankton from the experiments may be counted for radioactivity, and the carbon assimilation rates estimated.

It is the intention of the agency to commence work in June 1958. At present, it is impossible to state the exact cost of these operations. It will be possible either, to purchase these ampoules alone, or ampoules in connexion with filters which are to be counted at a later date. Filtration apparatus will be provided on request and at cost price. It should be noted that this agency will be organized on a non-profitable basis.

Report of the Committee on Terms and Equivalents

Dr. D. H. Cushing (Chairman), Mr. George F. Humphrey, Dr. K. Banse, Mr. Taivo Laevastu

There are certain general terms which need definition. They are:—

Standing stock = the quantity of plants or animals (at a given time).

Production = the quantity produced (the standing stock multiplied by the turnover rate).

Turnover rate = $\frac{\text{production}}{\text{standing stock}}$

Primary productivity = the carbon "fixed" per m.^{2} or per m.^{3}/unit time. (Note: measurements should be taken right through the photic zone.)

Gross primary production = the quantity of carbon taken up into organic combination. To obtain the net primary production, a correction must be made for respiration; to make this correction, the gross primary production should be calculated from sunrise to sunset and the loss by respiration, calculated for a 24 hour period.

Fertility is a vague term, which should be abandoned.

Each of the terms given above can also be expressed as energy. Again, we should always keep clearly in mind that whereas primary production is the quantity of algae produced, the word "production" may have other meanings, for example, the quantities of organic matter produced at higher trophic levels.

The following common definitions are suggested:—

Biomass (syn. live weight) = wet weight, including shells.

Displacement volume (or displacement weight, assuming that the S.G. of plankton is unity) = volume of fluid displaced by plankton which has been drained of water. (Method should be specified.)

Settling volume = settled volume of plankton, which includes variable quantities of interstitial water. (Method should be specified.)

Dry plankton = plankton dried to a constant weight. (Method of drying should be specified.)

Dry organic matter = dry plankton less ash.

Cell volume = volume of (one) cell.

Plasma volume (or volume of living matter) = cell volume less skeleton and vacuoles.

Equivalents are to be used with care. The following are recommended as being useful for phytoplankton:—

Carbon, dry organic matter, dry plankton, oxygen equivalent, and biomass.

Counts of phytoplankton cells should be accompanied with data for conversion to weight or volume.

The relationship between chlorophyll and total organic matter is variable and therefore chlorophyll should be used with caution as a measure of phytoplankton standing crop; the use of chlorophyll as measure of productive capacity is not yet proved.

Protein (or nitrogen) should be used with caution because its relationship to total organic matter is variable.

The following equivalents are recommended for zooplankton:— carbon, nitrogen, phosphorus, protein, dry plankton, biomass, and displacement volume. Protein varies with fat content. Nitrogen varies with chitin and protein content. Numerical values of the equivalents are given in the following tables.

Table 1. Phytoplankton equivalents

	Carbon 1 mg.	Dry organic matter 1 mg.	Oxygen equivalent 1 ml.	Plankton biomass 1 mg.	Dry plankton 1 mg.
Carbon, mg.	1	0·43	**0·53**[1])	0·024	**0·30**
Dry organic matter, mg.	2·3	1	1·2	0·055	0·69
Oxygen equivalent, ml.	1·9	0·83	1	0·046	0·57
Plankton biomass, mg.	42	18	22	1	13
Dry plankton, mg....	3·3	1·4	1·8	**0·08**	1

[1]) Bases for computation.

Table 2. Zooplankton equivalents

	Carbon 1 mg.	Nitrogen 1 mg.	Phosphorus 1 mg.	Plankton biomass 1 mg.	Dry plankton 1 mg.	Displacement vol. 1 ml.
Carbon, mg.	1	6·0	75	0·12	**0·60**	96
Nitrogen, mg.	0·17	1	13	0·020	**0·10**	16
Phosphorus, mg.	0·013	0·078	1	0·0016	**0·008**	1·3
Plankton biomass, mg.	8·3	50	620	1	5·0	**800**
Dry plankton, mg.	1·7	10	130	**0·20**	1	160
Displacement vol., ml.	0·010	0·060	0·75	0·0012	0·0060	1

Note: Dry organic matter in *zooplankton* is very nearly equal to dry plankton.

Table 3. Size of the plankton

Grouping of plankton by size.

Name of the size class	Approximate size limits	Characteristics by species composition
Megaloplankton	> 1 cm.	Large organisms like squids, salps, etc.
Macroplankton	1 mm.-1 cm.	Large zooplankton organisms
(Mesoplankton)	0.5-1 mm.	Small zooplankton organisms and their nauplii, large diatoms
Microplankton	> 60 μ.	Most phytoplankton organisms
Nanno	> 5 μ.	
Ultra	< 5 μ.	Bacteria, small flagellates

A. 1.

Counting Methods for Determination of the Standing Crop of Phytoplankton

By TRYGVE BRAARUD

The rôle of population studies in investigations of organic production

Numerical estimates of the actual population of the sea water does not give any direct information concerning the primary production of a water mass at the time of sampling. Such data of the plant population, however, are essential for obtaining a closer insight into the dynamic aspects of production, the cause of the geographical and seasonal changes in the photosynthetic activity within the euphotic layer and in the formation of new protoplasts.

What stress should be laid upon inclusion of studies of populations in investigations concerned with the quantitative estimation of production may be subject to discussion.

The introduction of the ^{14}C method has provided facilities for an extensive study of the potential production under a unit area of the sea at the moment of sampling. We are at present going through a period of collecting extensive material of such records, from various regions and different seasons. On the basis of these observations a correlation can be made using hydrographic data and observations on nutrients in order to draw up the broad lines of the interrelationship between the recorded production values and the environmental background. This can be done without making any observations of the organisms which are responsible for the photosynthetic activity.

After this first period the further exploration of the process of organic production in the sea will call for an intensification of the study of the productive organisms. The results which have been obtained on the biogeography, ecology, and physiology of the phytoplankton, both in general and for various species, will then form a useful basis. A combination of these studies with observations by means of the ^{14}C method should prove to be most fruitful.

Even during the first period knowledge of the phytoplankton distribution and its annual cycle within a region would be most valuable for a rational planning of surveys of production.

In this work it is essential to have at hand a method for quantitative studies of the population which is as satisfactory and requires as little work as possible.

Available methods

The marine phytoplankton includes organisms of very different sizes, from the larger diatoms and dinoflagellates down to very small forms. This is the reason why it is difficult to develop methods which yield satisfactory data on the whole plant community, the larger forms being present in relatively small numbers as compared with the smaller forms, even at the times of their maximum abundance.

The blue-green algae with a specific gravity lower than that of the sea water represent a special problem in quantitative plankton studies, but it will not be discussed here as this group is not of general importance in the sea.

The various methods for quantitative phytoplankton studies may be referred to the following categories:—

1. Net methods
2. Centrifugation
3. Filtration
4. Sedimentation
5. Direct counts
6. Culture methods

We may briefly consider the advantages and disadvantages of each of these categories.

Net methods

The advantage of net hauls is the ease by which large volumes of water may be filtered so that organisms which are present in relatively small numbers may be sampled. The main disadvantage is the exclusion of the forms which pass through the meshes of the net and the distorted picture which is often obtained of the relative abundance of the larger forms due to clogging. Even in the case of the new nets which have smaller meshes than the old ones there are important groups too small to be retained.

Although various types of devices have been described for measuring the amount of water which is being filtered, in many cases uncertainty prevails as to the reliability of the values obtained.

In conclusion it may be stated that *net methods should not be employed for quantitative phytoplankton studies.*

Centrifugation

Lohmann's (1908) centrifuge method was discarded for use in phytoplankton surveys when the sedimentation method was introduced. The objections raises against centrifugation by Allen (1919) and Steemann Nielsen (1933) are still unchallenged. The more favourable results obtained by centrifugation of live naked forms (Ballantine, 1953) may be due to the special adhesive nature of these objects, as indicated by Steemann Nielsen (l.c.) and cannot be relied upon when the method is used for other forms, especially in a preserved state.

Centrifugation has the advantage that the volume of water examined may be varied easily within a wide range. This method, therefore, may be useful in culture work when a comparison with other methods has shown that it is satisfactory for the special object in question, such as with naked flagellates.

Filtration

The older methods of filtration through sand and paper filters have been replaced by the more effective filtration through new types of filters. In the case of the molecular filters (Goldberg, Baker, and Fox, 1952) they retain even the smallest phytoplankton organisms. In the latter form filtration has the advantage of having all size groups retained and ease in varying the volume of the sample filtered. The main disadvantages of this method are the long time required to prepare the sample for counting and difficulties in identification of the small as well as many of the larger forms. This pertains to live as well as preserved material. The large forms cannot be turned over for closer inspection, while the small naked forms are often destroyed when filtered alive or subjected to great changes by preservation.

Sedimentation

The sedimentation method of Utermöhl (1931) with the use of an inverted microscope has proved to be a useful method for estimating quantitatively the intermediate size groups of the phytoplankton. For the smallest forms Utermöhl has described a special procedure of sedimentation in a Kolkwitz cell and examination of a minor fraction of the sediment at high magnification, a method which has been used with success by Rodhe (1955) for fresh-water samples. In both cases the disadvantage is that only preserved samples can be studied. As no preservative satisfactory for the small as well as some of the larger naked forms has yet been found for sea water, these are often inaccessible for identification. For the larger species the volumes which are commonly used are too small to give adequate representation.

The Haller Nielsen apparatus (1950) for a closer examination of forms which are not easily identified in the inverted microscope and the modification of the sedimentation cylinders so that sedimentation of larger samples is facilitated (Fraser, 1954) represent recent improvements of the method.

Direct counts

Only in exceptional cases can direct counts of the population give any adequate information on the total population. The method developed by Millar (Ballantine, 1953) using dark field inspection may give reliable figures for various size groups, but it cannot be recommended for general studies of phytoplankton populations as identification is not feasible.

Culture methods

Assessment of the number of autotrophic forms by means of dilution of the sea-water sample in culture media and observation of the subsequent growth has been carried out and this method has been used for checking the results obtained by other methods (Allen, 1919; Ballantine, 1953). The method is too laborious to be adopted for general studies of phytoplankton populations.

The conclusions which may be drawn from this brief review of the various categories of methods now in use are:—

(a) By means of a single counting operation it is not possible to have all the requirements satisfied which ought to be fulfilled if one should be fairly certain of obtaining an adequate record of all the autotrophic species occurring in the plankton.

(b) Net haul methods or direct counts should not be employed.

(c) The sedimentation method with the use of inverted microscope may be regarded as the most satisfactory standard method for obtaining quantitative records of the phytoplankton, except for the smallest groups. Attention, however, should be paid to the recent improvements of the method. Some large forms may not have been recorded in a satisfactory way when the largest volumes examined were 50 ml. or less.

(d) The most obvious obstacle in enumerating the total phytoplankton population of sea water is the lack of a suitable preservative for general use and for small forms, like the naked chrysophyceans and other naked flagellates. In the future special regard should be paid to the smallest species, motile and non-motile μ-forms, which hitherto have been neglected in general studies of marine phytoplankton.

Which procedure can be recommended for enumeration of the total population?

In view of the large amount of work involved in any enumeration of the total phytoplankton population of a water sample a research programme should be set up to consider the methods now in use in order to establish the least time-consuming and still satisfactory procedure and to find adequate preservatives.

For discussion during the Symposium the following procedure for enumeration of all size groups according to the principles laid down by Utermöhl (1931) may be suggested:—

1. The volume of the sample drawn from the water-bottle at sea should be increased to 300 ml.
2. Counting of the species belonging to the intermediate size groups, down to 5—10 μ and including the coccolithophorids, in sedimentation samples of 2 (1) ml. and 50 (25) ml. of preserved sea water by means of the inverted microscope (or by the modification introduced by Lund, 1951).
3. Counting of the largest diatoms and dinoflagellates in corresponding samples of 200 ml.
4. Counting of the smallest forms in a Kolkwitz cell with the use of ordinary microscope and water immersion objective as used by Rodhe (l. c.). Only a small fraction of the field is examined.

This procedure would require 4 different counting operations and would be very laborious.

In case a somewhat more time-saving procedure has to be employed, it would seem better to reduce the volume of the samples examined than to exclude any of the counting operations.

It should be underlined that enumerations according to this complex procedure should only be employed in cases when hydrographic observations from the same points where the water samples for phytoplankton studies have been drawn are available and the whole plan for the survey is satisfactory from the point of view of a phytoplankton study. In many cases a cruder method which gives only a very rough idea of the population may be not only justified but appropriate.

The method for enumeration should in any case be chosen for the purpose of the special investigation, taking into account both the accuracy of the method and the time required for counting. Doubtless, this point has been disregarded in many cases of marine survey work because of the tendency to apply methods which are familiar to the person organizing the survey.

When attempting to find a satisfactory preservative for sea-water samples two points should be given preference: 1) to provide a satisfactory preservation of the naked forms without destroying the calcareous coccoliths of the coccolithophorids, 2) to find the concentrations needed for hindering bacterial activities even at fairly high temperature, so that samples can be stored for a longer period of time without being destroyed.

Concluding remarks

In view of the scarcity of specialists in marine phytoplankton it may seem as if the requirements for population studies in connexion with the study of primary production in the sea, suggested above, are too rigid. However, as so little is known of the relative importance of the various size groups for the joint photosynthetic activity in a water mass, it is at present important to take all size groups into account when population studies are undertaken for use in studies on production.

This does not in any way impede the value of botanical studies of phytoplankton populations by means of methods which from a production point of view would seem unsatisfactory as they pay regard to special fractions of the population only.

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A. 2.

Chemical Methods of Estimating Standing Crop of Phytoplankton

By J. Krey

I. General Remarks

In many cases it is necessary for marine biologists to know the total quantity of living phytoplankton in a certain volume of water. Lohmann (1908) has solved this question by microscopic enumeration, followed by involved calculations of the plasma volume of each component of the phytoplankton. His method takes very much time and can be used only by experts on phytoplankton. One great advantage of this method lies in the true biological picture of the analyses given by it, covering the whole range of phytoplankton down to the smallest phytoplankton organisms of $2\,\mu$ and less. The number of samples which can be treated in this way is naturally very limited. Therefore a method which gives quickly the total quantity of phytoplankton is desirable, directly or by the way of a representative part. Microscopic examinations for completing and supplementing the information obtained in this manner is always necessary. In offshore and coastal waters we observe with rare exceptions a mixture of phytoplankton, zooplankton, organic and inorganic detritus in changing proportions. This fact makes it in most cases quite impossible to determine separately these various components by the methods generally used, e. g., the determination of total organic substance (by ignition loss or wet combustion), or dry substance, particulate phosphorus or nitrogen or albumen. These methods can only be applied together, giving the proportion of phytoplankton in the total particulate matter. We have to look out for specific components, representative of the phytoplankton, for example an amino acid. On the other hand, the quantities present in marine phytoplankton are small, so small that many difficulties are involved.

Several conditions have to be fulfilled:—

(i) The component to be analysed must be highly specific for the total phytoplankton.
(ii) The methods of analysis have to be sensitive enough for work with samples of sea water not exceeding 10 litres, with an accuracy of at least $\pm 10\,\%$.
(iii) The samples must, to some extent, be concentrated, conserved, and prepared for further analysis on board ship.
(iv) The work mentioned under (iii) must include the complete population of phytoplankton down to $1\,\mu$.

At present we only have a few methods to determine the plankton pigments, which are responsible for the assimilation. Perhaps it will be possible in the future to find an amino acid or an enzyme specific for the living phytoplankton which can be analysed fairly rapidly with sufficient sensitivity.

Until this problem has been solved, we have to be satisfied with methods of determination being less specific. The only one in general use in marine research is the determination of chlorophyll on the basis of its colour. The other pigments of phytoplankton are less specific than chlorophyll and of no great use in this regard. Many different procedures have been developed for chlorophyll determination following the original method for marine material, as described by Kreps and Verbinskaya (1930). In the course of time much criticism has been advanced on the principles of chlorophyll determination. In the following we will try to find out the real core and shall give some lines of procedure for several types of equipment.

The aim of every chlorophyll determination is to give, as precisely as possible, a portion of the whole standing crop of all living phytoplankton organisms. Principally we know that one unit of living chlorophyll under different circumstances is able to assimilate different quantities of CO_2. If we take the chlorophyll content as a whole, we should first test this in a natural population on its potential assimilation. But this being very difficult in serial work in the ocean, we have to postpone the study of this question. Parallel investigations on the chlorophyll content, the assimilation potential and the organic substance of phytoplankton in ecological different areas would give the general representative value of chlorophyll. In the following we shall bring some figures into consideration which may give us the range of order of chlorophyll content and of the activity of chlorophyll.

1. The chlorophyll content of phytoplankton

Since the development of the methods of chlorophyll determination, many valuable figures have been reported in regard to the percentage of chlorophyll in the living matter of phytoplankton organisms. Banse (1956) summarized these different figures and as an average he gave 3—4 % of chlorophyll in the *organic substance* of phytoplankton. The variations are from 0·75 (calculated from Riley, 1941) to 11·8 % (Gillbricht, 1952) for diatoms. Atkins and Parke correlate size of plankton organisms and their chlorophyll content; this figure enables us to calculate, very roughly, the relation between stereometric volume and chlorophyll content, giving, however, only the size range. In Table 1 the relationship between size of organism and chlorophyll content as reported by a few investigators is given.

Table 1. 1 mg. chlorophyll is contained in:—

50	mm.³	*Chlorella*
86	„	*Chaetoceros gracilis*
560	„	*Thalassiosira gravida*
59	„	*Nitzschia closterium*
97	„	*Gymnodium* sp.
260	„	*Hemiselmis rufescens*
181	„	mixed phytoplankton (Gillbricht)
279	„	dinoflagellates (Gillbricht)
139	„	diatoms (Gillbricht)
47	„	phytoplankton (Riley)

The range of variation is tenfold and is presumably due to errors in the calculation of volume and actual differences in the specific chlorophyll content of the different species and ecological types. More intense work of this type is desirable; it is very probable that species of dinoflagellates change their specific chlorophyll content as well as the activity of their chlorophylls too.

2. Assimilation number, a physiological equivalent

Gessner (1946) thoroughly studied assimilation numbers of freshwater phytoplankton. The assimilation number AZ is defined as follows:—

$$AZ = \frac{CO_2 \text{ in grammes assimilated per hour}}{\text{chlorophyll contents in grammes}}$$

Willstaetter and Stoll calculated AZ to be 6—9 under favourable conditions, Gessner's values range in surface samples from 6—13 and from 1—11 in 10 m. depth under constant light conditions of 10,000 lux. Manning and Juday report from Wisconsin lakes values from 3·3 to 19·2. Under constant light conditions in the euphotic layer these figures may give a relatively good equivalent for the photosynthetic potential of the chlorophyll. The differences found may be explained by a varying part of "dead chlorophyll" from the detritus, which could not be separated. In the case of vertical differences of AZ as mentioned above, especially occurring in phytoplankton living under the discontinuity layer, the adaptation of phytoplankton to low light intensities may be responsible for low AZ figures. On this point further laboratory research work has to be done on the assimilation potential of different species of phytoplankton.

We may conclude that chlorophyll generally represents the size of order of total phytoplankton substance and its assimilation potential. In the case of limited research work in a relatively homogenous area we may find a much closer correlation between chlorophyll and photosynthetically active phytoplankton.

From the investigations of Harvey (1950) and Gillbricht (1952) it is known that a great proportion of the particulate matter in the sea consists of detritus, which contains chlorophyll or its derivatives. In his review Vallentyne (1957) refers to chlorophyll in seston. He is of the opinion that the data at present available are too limited to warrant a general application of the chlorophyll measurements to represent the standing crop of phytoplankton. He further underlines the rôle of sedimentary chlorophyll degradation products taken into consideration in coastal waters with high turbulence.

Some difficulties arise from the existence of several different chlorophylls and their derivatives in the living phytoplankton and of other plankton pigments playing a certain rôle in the physiology of assimilation. If we extract our material, we always get a mixture of very different substances which have to be separated. The most convenient method to find out the quantity of chlorophyll in this mixture is the optical determination. All other techniques, such as chromatography or determination of chlorophyll magnesium gives too much trouble for serial work with very low quantities in phytoplankton research.

Before discussing the special methods of chlorophyll determinations on marine material it is necessary to give a short outline of the principles of optical measurements as well as of the general properties of chlorophyll.

3. Principles of optical measurement of concentration

It is desirable that the coloured substances extracted from plankton material follow the law "Lambert-Beer", i. e., that the extinction ($\log. I_0/I$) is in straight correlation to concentration and to the pathlength of the light. Optical measurements may be made in several ways, which are in principle different. They reflect the historical progress of analytical technique. Colorimetrical methods are characterized by the comparison of one distinct intensity of colour, for example in a standard range of test tubes, or with different heights of test solutions (e. g., Hehner cylinder, "emergence" colorimeter). It is necessary that the quality of colour of samples and tests is quite the

same, otherwise the comparison becomes inaccurate. In the case of a mixture of two substances with different colours to be investigated, we may take two of the "emergence" tubes, each filled with the pure solution and each to be changed in the height of each column separately (e. g., Dubosque double-colorimeter). The spectral quality of the light source used is not of any importance to the calibration curve. Colorimetrical methods are accurate to 1 %.

In spite of the difficulties in preservation of standard solutions it seems to be of some interest to undertake methodical investigations on a colorimeter for use in field work under simple conditions.

Photometric measurements do not compare the intensity of any colour, but they give the extinction of light; by means of a blend the light is softened, so that the two light rays, passing the solution and the blend, are equal in intensity. It is necessary to use a light source of high stability and optical filters of very small optical width. A difference in the voltage of the light source of 10 % gives an error of photometrical determination up to 2 % (Kortüm, 1955). If there is a difference in the optical centre of the filter and the maximum of absorption of solution, we get a difference in the observed colours. On the other hand the width of the filter has to be wide enough to give sufficient light. At different occasions the author working with the "Pulfrich-Photometer" observed differences in colour in the estimation of chlorophyll, in spite of the narrow optical filters of this apparatus. Instead of optical filters a monochromator may be used, the great advantage of which is evident, when combined with a strong light source and a small slit width. The accuracy of these photometers is limited by the sensitivity of our eyes and is about 1 %.

In order to get a higher sensitivity and accuracy many types of photoelectric photometers are developed. Using an electrical substitution method and colour filters or monochromators an accuracy of about 0·2 %—0·1 % has been reported.

4. General remarks on chlorophyll

In order to give general properties of chlorophyll we follow the reviews of Smith and Benitez (1955) and Stoll and Wiedemann (1952). Today we distinguish the chlorophylls *a*—*e* and further protochlorophyll and bacteriochlorophyll with similar light absorption in the red part of the spectrum. The molecular weights of the main chlorophylls and their derivatives are as follows:—

Chlorophyll *a*	893·48
Chlorophyll *b*	907·46
Pheophytin a	871·18
Pheophytin b	885·16

The chlorophylls may be dissolved in alcohols, ether, acetone, and other solvents.[1]) Their specific absorption coefficients

$$\text{Ek} = \frac{\log. I_0 - I}{c \times d^{2})}$$

are given for the red maxima in ethereal solution together with the corresponding fluorescent maxima (Table 2).

The figures of the absorption coefficients are highly dependent on the purity of substances and on the specific properties of the spectrophotometer, particularly the width of the spectral band.

Table 2

Absorption maxima, absorption coefficient, and fluorescent maxima in the red part of the spectrum for different chlorophylls and pheophytins (these in brackets) in ether

	Red maximum		Absorption coefficient maximum		Fluorescent maximum	
Chlorophyll (Pheophytin)						
a	666	(667)	100·0	(63·7)	666	730
	688		1·5		(672·5)	(715)
b	644	(655)	62·0	(42·1)	649	708
					(657)	(707)
c	628	(641)	22·0	?	635	690
		(689·5)			(649)	(7 9)
d	688	(692)	110·4	(82·7)	693	750
	643	(631)	14·3	(7·75)	(701)	
	663		11·3			
Protochlorophyll.	623	(638)	36·9	(2·5)	629	(—)
Bacteriochlorophyll	697	(680)	10	(12·0)	695	in alcohol
	660	(625)	6	(4)		

The maxima generally cover a very small range of the spectrum and the band width should not exceed the width of the specific maximum. It is desirable always to indicate the band width in order to make comparisons possible.

The absorption maxima of the different chlorophylls are quite close in the red part so that an optical influence of one substance on the other is probable, especially when the band width is great. The determination of two different chlorophylls in one solution may be given by absorption measurements at two different wave lengths. Smith and Benitez (1955) give an example of such a calculation for a mixture of chlorophyll *a* and *b*:—

$$D_{662} = 100{\cdot}9 \times C_a + 6{\cdot}2 \times C_b$$
$$D_{644} = 15{\cdot}8 \times C_a + 62{\cdot}0 \times C_b$$

where $D = \log. I_0/I$ and C_a and C_b the concentration of chlorophyll *a* and *b*.

The maxima of absorption vary slightly with the solvent as shown for chlorophyll *a* and *b* (Table 3).

[1]) By mixing with water (10-20 %) the solubility of disturbing substances is depressed.

[2]) c = concentration (g./l.),
d = diameter of absorption vessel.

Table 3. Maximum of absorption of chlorophyll *a* and *b* with different solvents (Harris and Zscheile, 1943)

Solvent	Chlorophyll *a*		Chlorophyll *b*	
	max. red	max. blue	max. red	max. blue
Methanol	664	434	651	471
Butanol	665	432·5	—	—
Ethylether	660	429	642·5	453
Acetone	661·5	431·5	643·5	456
Carbon tetrachloride	663·3	432·5	644	457
Benzene	664	432·5	645	459

The influence of the solvents is far more important when comparing the specific absorption of the red and those of the blue maxima as done by Harris and Zscheile (1943), who took $D = 0{\cdot}165$ at $505\,m\mu$ (Table 4).

Table 4. Relation of absorption coefficient of blue maximum/red maximum for different solvents

Solvent	Methanol	Butanol	Ethylether	Acetone	CCl_4	Benzene
Chlorophyll *a*	1·00	1·03	1·33	1·26	1·32	1·33
Chlorophyll *b*	2·85	—	2·98	2·95	2·54	2·45

From the purely optical point of view methanol is preferable, because its blue maximum absorption is almost the same as its red one in contrast to the other solvents mentioned. On the other hand, the differences in the position of the red maximum between chlorophyll *a* and *b* are relatively small compared with other solvents. As will be seen later on, chlorophyll *b* is present only in very small amounts in marine phytoplankton. For this reason the above-mentioned difficulty is not serious, which is also evident from Table 5.

From this table we may conclude that even if the same quantities of chlorophylls *a*—*c* are mixed in the same solution, the disturbing influence of chlorophyll *b* and *c* on the estimation of chlorophyll *a* is of minor importance.

Strain (1951) has reported on the occurrence of chlorophyll. The special problems regarding marine phytoplankton are discussed by Richards.

The question of the optical and chemical stability of chlorophylls is of relevance for all methodological developments. Zscheile, Comar, and Harris propose conservation of undestroyed plant substance at — 20° C. without drying the material. The use of free acids, which remove magnesium from chlorophyll and form pheophytin, is to be avoided. In the course of formation of pheophytin the colour of chlorophyll *a* changes from blue-green to olive-green, that of chlorophyll *b* from yellow-green to bordeaux-red (Stoll and Wiedemann, 1952). According to Smith and Benitez the formation of pheophytin gives only a slight change in the red absorption maximum but a strong one in the specific absorption coefficient in the maximum (see Tab. 2). At any rate it is of some advantage to avoid the formation of pheophytin.

Table 5. Specific absorption coefficient at some given wave lengths (Smith and Benitez)

Wave length	662	643	644	628	624 $m\mu$
Chlorophyll *a*	100·9	—	15·9	—	14·2
„ *b*	6·2	—	62·0	—	11·0
„ *c*	—	—	—	(22·0)	—
„ *d*	11·3	14·3	—	—	—
Protochlorophyll	0·2	—	1·0	—	39·9

Table 6. Main pigments in algae (Strain)

Chlorophyll	*a*	*b*	*c*	*d*	*e*
Bacillariophyceae	+++	—	+	—	—
Chrysophyceae	+++	—	?	?	?
Dinophyceae	+++	—	+	—	—
Cyanphyceae	+++	—	-	—	—

(+++ very common, + present)

Chlorophyll is also decomposed by the enzyme chlorophyllase, which splits up the molecule. The activity of this enzyme has to be destroyed by poisoning, short heating, or intense cooling.

Furthermore, the sensitivity of chlorophylls to light is well known. The samples have to be stored in a dark room and analyses have to be carried out in soft light.

II. Special Procedures for Measurement of Chlorophyll

The procedure may be divided into several steps, each being variable and to be adapted to the special circumstances of the material under examination. There are many parallels to limnological methods and some specific differences too, caused by the low concentration of chlorophyll.

1. Collection and concentration of material

Although this is a general problem for all other methods of estimating the standing crop, some remarks on this subject are essential. Every investigator will try to get as accurate results as possible. In the case of chlorophyll determinations this means that he has to take the entire population of living phytoplankton from the great colonies, visible to the naked eye down to the small phytoflagellates of $1\,\mu$ size. On the other hand, it is obvious that this is very time-consuming, especially in regard to the quantitative haul of the smallest size organisms.

The first methods to gain phytoplankton material for extraction of chlorophyll were those of towing nets through a definite column of water, sometimes controlled by flow meters, thus giving the exact volume of water filtered through the nets (Kreps and Verbinskaya, 1930; Harvey, 1934; Riley, 1938; and Ramamurthy, 1953). Methods of towing with nets have some advantages: one gets relatively much material for extraction and this allows the use of an optical method of lower sensitivity. Further, a

net towed through the water partly eliminates the natural heterogeneity of plankton distribution observed as well in coastal as in offshore waters. On the other hand, this may also be a disadvantage, when the investigator follows problems of vertical distribution. But there is an important source of error: the net has only pores down to about 40 μ. All organisms under this size escape and the question arises whether these organisms occupy a significant volume of the phytoplankton. Many investigators have studied this question (Lohmann, 1903, 1908; Atkins, 1951; Goldberg, 1952). Harvey (1950) investigated the filtration efficiency of paper and membrane filters. Using membrane filters of an average pore size of 0·9 μ in coastal water he obtained about two times as much green colour as with filter papers. Also in offshore waters the error may be too great to allow sampling with plankton nets. From the countings of Hentschel during the "Meteor" Expedition 1925—1927 (taken with the centrifuge) we may give two examples:—

Stereometric volume of phytoplankton

Station 60	0 m.	Station 247	0 m.
Mainly diatoms		Mainly dinoflagellates and coccolithophorids	
$> 40\,\mu$	6.15 mm.³		0.12 mm.³
$< 40\,\mu$	3.42 mm.³		0.03 mm.³

Steemann Nielsen (personal communication) refers that not only in coastal areas but also in the open warm ocean the phytoplankton $< 40\,\mu$ may make out nearly 100 % of the total (in average 94 %). So we may conclude that it is very dangerous in many cases for quantitative chlorophyll determinations to work with the plankton net without observing microscopically the size composition of phytoplankton in sedimented samples.

In contrast to net-towing the use of a centrifuge or analytical filtration in most cases permits the study of sections of the water column. In recent years centrifuging of samples was shown not to be sufficient compared with filtration or combined sedimentation and filtration through a very fine paper or collodion filter (Richards, 1955; Atkins, 1939; Krey, 1939; Graham, 1943). A great variety of filtration methods are in general use so that detailed description is not possible here. Attention is however, drawn to a semi-automatic method described by Krey (1949), which allows filtration up to 5 litres without necessity of supervision. For many purposes the method employed by Atkins (1951) will be of some interest. Atkins lowered the pH of sea water to 8 by addition of dilute acetic acid and then treated 1 litre of this sea water with 5 ml. of a 1 % solution of $KAl(SO_4)_2$. The precipitate includes all the particulate matter, in particular the very small phytoflagellates and the detritus. Some time later the clear supernatant was decanted and the residue filtered through a fine filter paper. Korringa and Postma (1957) working in the Gulf of Naples, separated the suspended matter by filtering first the water through the finest bolting silk with meshes of 60 μ width, thereafter the filtrate through the finest filter paper (Delta 385 or Schleicher and Schüll 1575). They compared the results obtained by filtration through a coarse filter paper (Delta 365) and a fine one. In regard to the accuracy to get the entire phytoplankton population, the filtration method is superior to net-towing and centrifugation. On the other hand it gives only relatively small quantities of chlorophyll for subsequent optical determination, because the volume of water filtered is limited. So these methods necessitate an optical method of sensitivity higher than those mentioned previously.

2. Dissolving and preserving chlorophyll

From the wet material obtained during the preceding manipulations we have to get a solution of chlorophyll as complete and as quickly as possible. Furthermore, no derivatives of chlorophyll should be produced by the process of extraction, because the optical absorption maxima are different from those of pure chlorophyll. The solution of chlorophyll has to be stable at least for several days, and preferably several weeks.

Unfortunately we do not possess comparative investigations on these points especially for different kinds of marine phytoplankton. Most of the investigators use aqueous acetone as a solvent, so Riley (1946), Harvey (1934) 80 %, Graham, (1943) 80 %, Atkins and Parke (1951), Richards and Thompson (1952) 90 %. The addition of water to acetone lowers the simultaneous extraction of disturbing substances, according to Stoll and Wiedemann (1952, p. 549), who used 85 % acetone. The time of extraction is given by Richards and Thompson (1952) to be not less than 9 hours for 90 % acetone. After 24 hours they found no spectrophotometric evidence of decomposition and no increase of extracted chlorophyll by fresh portions of solvents. Gardiner (1943) controlled the course of the extraction with the microscope. Freshwater algae show an inhomogeneous extraction; diatoms were totally extracted, but Dinophyceae only incompletely.

Alcohols have also been used by different authors: Kreps and Verjbinskaya (1930) 96 % ethanol, Krey (1939) methanol, Kalle (1951) methanol, Stoll and Wiedemann (1952) alcohols. Zscheile, Comar, and Harris (1944) note that methanol is satisfactory for complete extraction of chlorophyll. To minimize pheophytin formation they propose the addition of $MgCO_3$ to the solvent. They found ether to be a good solvent, the solutions were stable for 4 weeks when kept cold. After that time pheophytin was formed. Chlorophyll solutions in ethylether have sharper absorption bands than those in acetone. Stoll and Wiedemann (1952, 1955) give examples for ether solutions. In spite of its many favourable qualities, however, its high volatility prevents a general use.

Two methods have been proposed to prevent the destruction of chlorophyll in solution:— 1) to destroy the enzyme chlorophyllase which forms pheophytin, by keeping the filter paper with the phytoplankton for a short time (up to one minute) into hot water

vapour (KREY, 1939), 2) the addition of $MgCO_3$, as mentioned above and applied too by RICHARDS and THOMPSON (1952), is to be recommended in order to prevent partial destruction. At any rate the solutions have to be stored in the dark, at low temperatures, and the laboratory manipulations have to be made in dim light.

3. Measurement of concentration

The colorimetric measurements of chlorophyll require only little equipment and are easy to carry out. HARVEY (1934) gives a simple procedure which is in general use today (RAMAMURTHY, 1953; MARGALEF, 1957), although many modern instruments (filter and spectrophotometer) allow a much higher degree of accuracy. A number of tubes with equal diameters is filled with different quantities of the standard solution. One pigment unit

$$\text{p.c.c.} = 25 \times 10^{-6} \text{ g. } K_2CrO_4 + 430 \times 10^{-6} \text{ g. } NiSO_4 \times 6\ H_2O$$

in faintly acid distilled water. The colour of this tube is compared with the greenish solution extracted from the net-towed plankton samples. In spite of the colour of the test tubes being chosen according to the depth of colour of the sample tube, the difficulty arises in comparing solutions with at least two different coloured components, because in the extract we find not only two or three chlorophylls, but also different carotenoids. The advantage of this method is its great simplicity. On the other hand the results should be better comparable with absolute quantities of chlorophyll *a*. Many authors tried to estimate the chlorophyll *a*-equivalent for the HPU (Harvey pigment unit). BANSE (unpublished) and MARGALEF, MUNOZ, and HERRERA (1957) have summarized the relevant data and found the range to be from 0·13—0·88 μg. chlorophyll *a* per 1 HPU.

ATKINS and PARKE (1951) used a colorimeter, the American Kober-instrument, similar to that of Dubosque. They avoided the differences in the quality of the colour between sample and standard solution by using a red filter glass (RG 1, Schott). As a standard they employed a commercial chlorophyll preparation. According to a personal communication from ATKINS, his early results made by means of a red filter should be neglected. In spite of all errors the principles, followed by ATKINS, may perhaps in future work lead to a simple double "emergence" colorimeter for work with a pure and stable standard solution of chlorophyll *a* with the elimination of colour differences.

In order to avoid the difficulties arising from the Harvey method the author developed a photometric variation (KREY, 1939). The absorption of the methanol extracts was determined in a Pulfrich photometer and later on in an electrical colorimeter (Elko II, Zeiss) working with filters (Table 7).

The method gives a sufficient sensitivity of about 0·1 μg. chlorophyll. The B_{hw} is relatively great with S 66 and may cause an error in method, e. g., when

Table 7

SW: effective filter intense point in mμ. B_{hw}: effective half-value band width in mμ.

Filter	S 66 665	32
	J 66 665	5–10

much chlorophyll *b* or *c* is contained in the extract. This error is much smaller with the new J 66 so that working with this is almost comparable, from an optical point of view with instruments, equipped with a monochromator prism. Because of the band width of 5—10 mμ with J 66 the specific absorption coefficient for chlorophyll *a* in its red maximum cannot be as high as determined with a monochromator instrument with only 2 mμ band width.

This method takes a medium position between the colorimetric and the spectrometric method and may give sufficiently satisfactory results, if handled with necessary precaution.

From a general point of view the spectrophotometric determination of chlorophyll will give the most precise results where chlorophyll has to be estimated in mixture with other coloured components. The progress of technics offers highly perfected instruments, e. g., Beckmann, Unicam, or Zeiss spectrophotometer. They will allow a small band width of 2 mμ and sufficient light intensity. The number of chlorophyll determinations made with these instruments in marine research is quickly mounting, so that we can give only a few examples here. Already in 1930 KREPS and VERJBINSKAYA gave spectrophotometrical determinations of chlorophyll, unfortunately without dealing with any details.

The most extensive study in this field has been done by RICHARDS (1953) and RICHARDS and THOMPSON (1953). They used a Beckmann DU spectrophotometer with a nominal band width of 1.6—2 mμ in the red part of the spectrum. They present the instrumental error for their equipment as follows:—

	Error ±20 %	10 %	5 % with following concentrations
Chlorophyll *a* mg./m.³ ...	0·16	0·32	0·64—12·8
„ *b* „ ...	0·25	0·50	1·00—20·0
„ *c* MSPU/m.³	1·09	2·18	4·36—87·2

As the specific absorption coefficient for chlorophylls is not yet exactly known, RICHARDS and THOMPSON replace it by the special term: pigment unit, which should be about one gramme. The abbreviation MSPU means about 1 mg.

RICHARDS and THOMPSON made their determinations with 90 % acetone extracts of plankton at 665, 645, and 630 mμ in order to get the three components and put up the following equations:—

$$D_{665} = 0{\cdot}0667\ C_a + 0{\cdot}0065\ C_b + 0{\cdot}0011\ C_c$$
$$D_{645} = 0{\cdot}0164\ C_a + 0{\cdot}0456\ C_b + 0{\cdot}0044\ C_c$$
$$D_{630} = 0{\cdot}0119\ C_a + 0{\cdot}0127\ C_b + 0.0104\ C_c$$

where D means log. I_0/I and C the concentration of the chlorophylls *a*, *b*, and *c*.

Solving these equations in the case of a practical analysis of a plankton sample they found:—

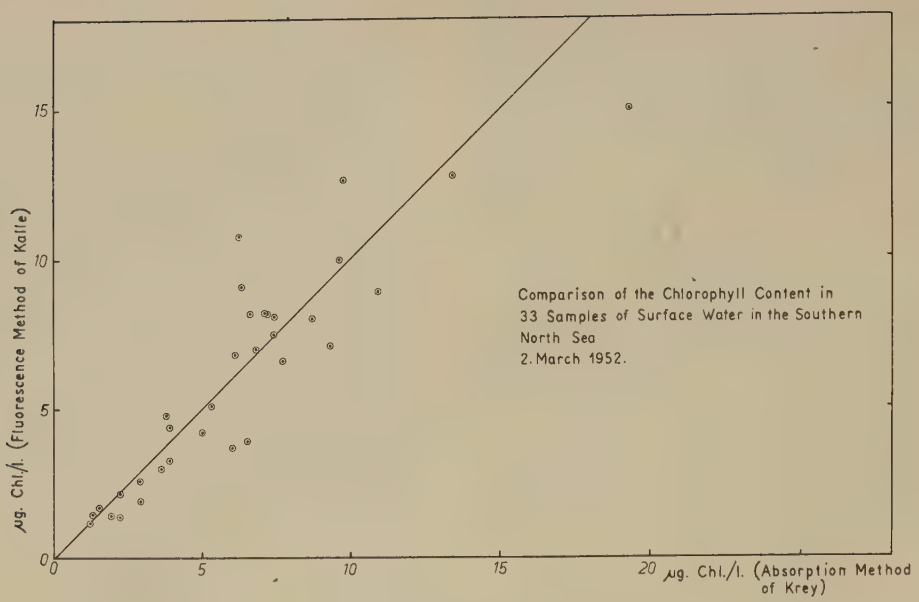
µg. Chl./l. (Fluorescence Method of Kalle)
µg. Chl./l. (Absorption Method of Krey)
Comparison of the Chlorophyll Content in 33 Samples of Surface Water in the Southern North Sea
2. March 1952.
0
5
10
15
20

Figure 1.

Chlorophyll *a* mg./m³. = 3.37
„ *b* „ = 0.07
„ *c* MSPU/m³. = 1·97

This short extract may perhaps show the superiority of the spectrophotometric method, permitting exact figures for the three components of chlorophyll. We may neglect for many purposes chlorophyll *b*, which takes only $< 2\,\%$ in this example as well as in many others. But chlorophyll *c*, ranging to about $^2/_3$ of chlorophyll *a* may not be overlooked. It will surely be necessary to recalculate many chlorophyll *a* determinations in order to get *total* chlorophyll. A general factor to calculate total chlorophyll *a* has to be found with the help of a good spectrophotometer.

The above equations also show that the determination of chlorophyll *a* is only in a very small degree affected by the presence of chlorophyll *b* and *c* under natural conditions. It may therefore be allowed to work in the future with an exact determination of chlorophyll *a* only in order to estimate total chlorophyll.

Kalle (1951) developed a highly sensitive method on the basis of *fluorescense measurements* of chlorophyll solutions. After extracting the filter residue from the filtration of 100 ml. sea water with only 1 ml. 85 % acetone for only 15 minutes he filled a microtube, containing only 0·7—0·8 ml. with this extract. In ultraviolet light the fluorescense of this was compared with a standard glass (GG 3, Schott) which showed the same fluorescense colour as the chlorophylls. In order to avoid blue fluorescense, disturbing the comparison of the two colours, he filtered the red fluorescense by a red RG 2 Schott filter. This is the most sensitive method we know up to date for working with samples of sea water. By the kindness of Kalle a comparison of this method with the absorption method of the author was made on 33 samples of surface water from the North Sea, taken from the same carboy and immediately worked up on board a ship. Figure 1 shows a sufficient agreement if we consider that the red fluorescense is caused by all chlorophylls (Tab. 2), the absorption measurements, on the other hand, can only give chlorophyll *a*. Working with small volumes of water as done here, we have to consider natural inhomogeneity in the samples.

4. Calibration

In order to get comparable values for all chlorophyll determinations it is of importance to introduce a standard solution of chlorophyll *a*. In oceanography the normal water has already been introduced for a long time for the determination of salinity. Stoll and Wiedemann (1955) describe a chlorophyll *a*, which is chromatographical pure and stable in the dry condition as well as in ether solution and this will be proposed as a standard substance.

5. Other chemical determinations

Although chlorophyll *a* is the representative substance of the highest specificity available today, some other less specific constituents will be mentioned. The methods of estimating these constituents may be applied on pure samples or, if samplers are controlled by microscopic investigations, on the portion of non-phytoplankton matter.

At present the determination of P, N, C, and albumen in particulate matter plays but a minor rôle in general planktological research work because of relatively complicated methods (vide: von Brand, 1935; Harvey, 1948; Kay, 1954; Krey, 1951, 1957). Their introduction depends as well on the problems under investigation as on the state of equipment.

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A. 3.

Suspended Matter as an Index of the Productivity of the Sea

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It is beyond doubt that the greater part of the organic production in the sea is primarily contributed by the phytoplankton. The amount and efficiency of the production by the phytoplankton and the physiology of these floating plants have been studied by many workers, and study will be further continued in this field. It should be noted, however, that the ratio between the production of phytoplankton taken by nets and the landing by fisheries (i. e., the commercial production) varies considerably in different sea regions. As has been confirmed by oceanographers, a large amount of organic matter is present in the sea water in the form of minute organisms, possibly playing an important rôle in the production cycle in the sea. This suggests the possibility that the amount of these organisms may be correlated with the commercial productivity of a sea area more closely than the production of phytoplankton taken by nets. Actually, a few to dozens of times as much organic matter is found in the sea in the form of microparticles retainable by the molecular filter (MF), as in the form of the phytoplankton taken by nets.

Table 1. Ratio of fisheries catch to phytoplankton production

Region	Fisheries catch / Phytoplankton production	Author
Caspian Sea	0·45 %	Bluerich 1936
Georges Bay ...	0·10 %	Clarke 1946
Tokyo Bank....	0·55 %	Hanaoka 1952
Lake Suwa.....	0·66 %	Hogetsu 1946

Methods and results

The distribution of the suspended matter under consideration is not homogeneous, particularly in coastal waters. The coefficient of variation of the measurements usually amounts to 100 to 300 % in the water samples taken successively at one station, or concurrently side by side with the ordinary water sampler. Therefore, any reliable measurement can be made only by dealing with such samples that are taken through a certain length of time so as to cover a larger mass of water. In one of our methods, the instrument shown in Figure 1 a is hung down in the water, and, with the aid of a vacuum pump on the boat, the sea water is filtered through the powder filter-plate (Fox, 1953) in the cup, the suspended organisms and particles being retained on the plate. About three litres of sea water can be filtered in 40 to 50 minutes by using two filter cups (3 cm. in diameter). And in the other method, a bottle is also hung down with two glass tubes, of which one is an ordinary tube, and the sea water runs into the bottle as the air in it comes out in bubbles through the capillary opening of the other tube.

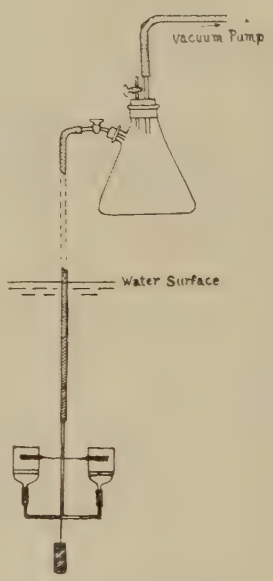

Figure 1 a.

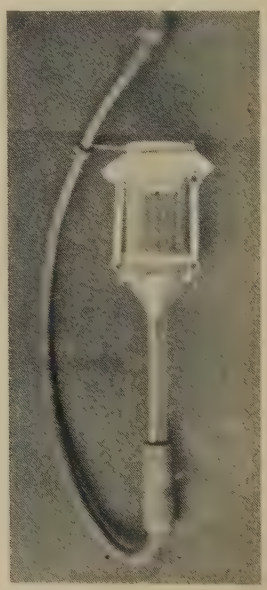

Figure 1 b.

Table 2. The amount of suspended matter

Particles retained by:	Sea region	Suspended matter dry weight, mg./m (inorganic + organic)	Plant protoplasm, dry weight, mg./m	P/S (%)	Date
Plankton net (XX 13)	Kanawa Strait	16— 86	1·0— 8·6	1·8—15·0	Dec. '56 to Mar. '57
	Konoura region	20— 112	1·7— 8·6	3·7—15·5	ditto
	Kasaoka Bay	67— 486	5·5— 18·6	3·4— 8·7	Feb. '57 to Mar. '57
	Kaida Bay	24— 76	1·7— 6·2	5·6—11·5	Dec. '56 to Mar. '57
MF (APD 0·4 μ)	Kanawa Strait	750—3700	26·5—149·6	1·3— 7·9	
	Konoura region	700—1500	36·5— 76·7	2·8— 7·4	
	Kasaoka Bay	1100—1860	20·0— 86·0	0·5— 1·8	
	Kaida Bay	670—3330	26·8— 82·2	1·6— 6·2	

The speed of water running into the bottle is controlled by the opening area of the capillary, so as to get the same rate as that of the first method. Sampled water is filtered through MF. The rate of filtration differs in different water masses.

It may be considered that almost all the particles originally present in the sea water are retained by the MF of APD (average pore diameter) 0·4 μ, for the extinction coefficient of the filtrate is almost the same as that of distilled water. No significant difference is found between the measurements of the suspended matter for the samples taken in this way at the bow of a boat and at the stern.

For the purpose of estimating the amount of plant protoplasm contained in the aforementioned suspended matter and in the net-collected plankton, the following measurements were made. The sea water is first strained through a plankton net of the mesh XX 13, and the filtrate is further filtered through the MF. The two fractions of the particulate matter thus obtained are respectively extracted in 90 % acetone, and the amount of chlorophyll *a*, *b* and *c* are determined with the spectrophotometer. By multiplying the amount of chlorophyll *a* and *b* combined with the factor 34·4, we obtain the dry weight of phytoplankton (Atkins and Jenkins, 1953) which is tentatively regarded as the dry weight of plant protoplasm.

The results of the measurement, shown in Table 2, clearly indicate that far larger amount of plant protoplasm is present in the form of the micro-particles retainable on the MF than in the form of larger particles to be retained with the plankton net. It is also indicated that the fraction of smaller particles far exceeds the one of larger particles in dry weight. On the other hand, the values of P/S in the table indicate that the fraction taken by net is richer in plant protoplasm than the MF-retained fraction, and that the P/S is usually lower than 20 %.

The contents of organic matter estimated from ignition loss were in the range of 6—51 % (20·2 % on the average) of the MF samples from Kasaoka Bay, 15—83·3 % (47·0 % on the average) in that from Kaida Bay, and 35·3—65·0 % (52·1 % on the average) in the Konoura region. They seem to be higher in the samples taken in the outer part of the secondary bay (Jones & Wills reported 63—73 % in Plymouth Sound water, 1953).

According to our recent study, the average size of suspended particles under consideration seems to be closely related with the distribution of filter-feeding clams and their nutrition.

The visible transparency depth T by Secchi disc has long been used as the simplest index of the amount of suspended matter and the underwater illumination (I_T) at the depth T was reported to be 9—22·6 % (Poole & Atkins, 1929), 8—29 % (Young, 1941), and 10·4—40 % (Hanaoka, Furukawa *et al.*, 1952—57) to the incident light at the water surface (I_0). And this percentage has been estimated to be 18 % on the average, and therefore uT is assumed to be a constant 1·7 in the equation $I_T = I_0 \exp.(-uT)$, where u is the extinction coefficient, on which various computations have been based in most of the studies on the optical character of sea water. However, such a wide variation as shown above may not be assigned to the observation error alone, although those variations have not yet been analysed quantitatively.

Our observations in many localities have confirmed that the ratio (I_T/I_0) takes a fairly definite value to each water mass and season, and the following equation has been obtained, that is, $I_T = I_0 \exp.(-aT^b)$ where b approximately equals to a constant, 0·7, showing no inconsistency in the range of our observations (inshore or bay water and neritic water) and this relation seems to be the case with the oceanic data by Kpt. The value of the coefficient a is determined by the character of the water mass.

The relation between T and I_T/I_0 can be recognized by experiments. Observing the underwater illumination I_d at every depth d and T in these various suspensions such as colloidal solution of Mn-oxide, rusted tap water, cutch extract, and indian ink, we obtained a clear relation $u = aT^{-b}$. And since there also exists the Lambert equation $I_d = I_0 \exp.(-ud)$, we obtain $I_T = I_0 \exp.(-aT^b)$. In these experiments a relation $u = kC^B$ (C is the concentration of the suspended matter) is also obtained, where B takes different values by suspensions, other than unity. In case of the suspended matter collected from sea water through the molecular filter, we have $u = kS^{0.2}$ where S is the concentration of the suspended matter (mg./l.), and k is approximately related to a in the range $a > 0{\cdot}3$ by $k = 0{\cdot}86a$—$0{\cdot}154$. Therefore, the concentration being kept constant, a gets larger as

the radius of the particles increases, that is, as the particles become more numerous in number. This means that the average size of the particles is smaller as the value is observed to be larger.

Table 3. Values of a and b in suspensions

Suspension	Radius of particle in μ	a	b
Colloid of Mn-oxide	+	4·47	0·05
Rusted tap water ..	0·2	2·44	0·11
Cutch extract	0·4	5·90	0·20
Indian ink	0·8	(1) 3·58 \ (2) 4·18 /	0·024
Chlorella	3·5	2·15	0·143
Sea water	?	0·13—2·00	0·70

Since another relation $S = \gamma\tau$ was observed, where τ is the absorption coefficient to the incident light, $a = 0{\cdot}92\, u\tau^{-0.2} + 0{\cdot}179$. From this equation a can be computed, given u and either S or τ. If an underwater turbidity meter is available, a will be obtained even in case where T cannot be observed or a is required by layer. Thus, a is competent to be an index of the particle size so far as b is kept constant. It can discriminate water masses very well and conveniently suggest the micro-structure of the sea.

Oysters show different meat characters and growth-rates by area even in a single bay. However, an interesting correlation has been found between their horizontal or seasonal variations and a-value. Generally speaking, the growth-rate and the meat quality of oyster are good in the region where a is 0·4—0·5, and not good where $a > 0{\cdot}7$, when the water content of oyster increases above 85 % with a semi-transparent appearance of meat as is so-called "Mizu-gaki" (water oyster). However, even in case of $a > 0{\cdot}7$, such a region of very much suspended matter as Kasaoka Bay seems to be a little better for oyster than the region of $a = 0{\cdot}4$ or 0·5 but a very little suspended matter showing $T > 10$ m. No oyster yard can be seen in the region $a < 0{\cdot}3$. These facts are supposed to be related with the total amount of utilizable organic matter for oyster.

Fishing grounds of "Mogai" (*Anadara subcrenata*) have the value 0·7—0·9 of a, and the pearl oyster farm is situated in general in the region of $a = 0{\cdot}3$ to 0·5 and pearls of best quality seem to be harvested in the region of $a = 0{\cdot}4$.

Relations of the suspended matter with the fisheries productivity

It may be reasonably believed that not only filter-feeders but also animal plankton and larvae live on these suspended particles. Recently it has been reported, too, that fish larvae take suspended particles of 20 to 30 μ in diameter. And the population size and production of fish can be supposed to be affected by the amount of these suspended particles. The following test may be said to be one of the materials for recognizing the relation.

It has been confirmed that a hyperbolic relation exists between the amount of suspended matter and visible transparency depth T by Secchi disc. But no relation is found between T and the displacing volume of net-plankton P in general, while the outermost points on the graph, which stand for the stations in the Kuroshiwo Current or its neighbourhood, are seen to fall on a hyperbolic curve. Assuming the amounts of suspended matter other than net-plankton to be

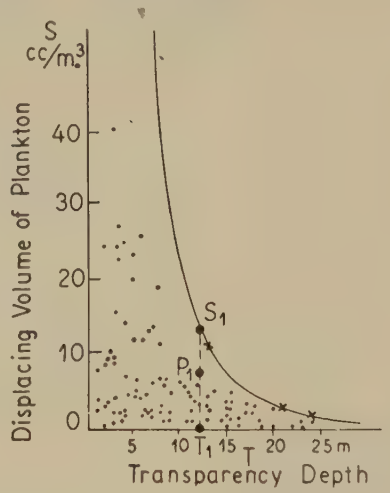

Figure 2. Relation between displacing volume of plankton and visible transparency depth.

The curve is expressed by $S = 8500\, T^{-2.64}$.

negligible in the water at these stations, this hyperbolic curve can be considered to be a standard one which shows the relation between P and T. Assume S_1 as a point on the standard curve intersecting the ordinate through observed T_1 and P_1 and we can suppose there is suspended matter in the water as much as the amount represented by the volume of net-plankton of $P_1\, S_1$ besides the observed net-plankton $T_1\, P_1$. (See Figure 2.) Thus we can consider a value S (segment ST) as an index of the total amount of suspended matter there. Denoting S for the yearly mean of a bay, we have obtained an equation $F = -1290 + 1930 \log. S$, where F is the fisheries products in wt./km². other than shellfish and weeds, and S is the grand mean of the means, weighted by area of equal S survey by survey (necessary are more than four surveys a year). This equation has been found to be applicable to more than ten bays studied around Japan, and also the value of F estimated by inserting to this equation the value of S based on data of oceanographical surveys in Seto Inland Sea area, where we have about 90 stations covered at least four times a year, has been seen to coincide with the actual landings estimated by the statistical Agency of the Japanese Government within 10 % of discrepancy. However, this relation can be applicable to the total area of a bay within the mouth open to the ocean, but not to parts of a bay or inland sea. It may be that the trophic composition

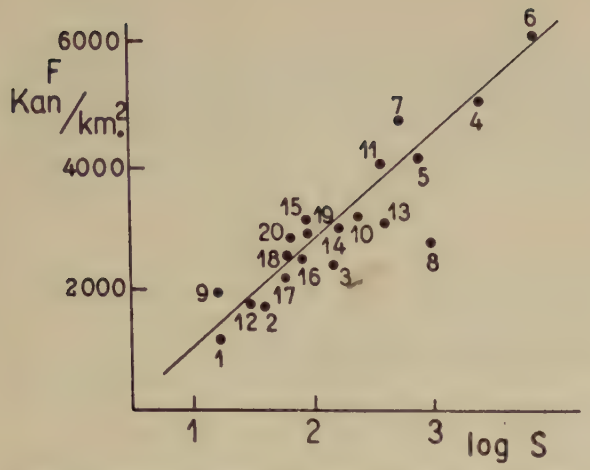

Figure 3. Relation between fisheries production wt./km.² and S.

The line is: $F = -1290 + 1930 \log. S$.

1. Mutsu Bay. 2. Kesennuma Bay. 3. Kamo-Lake. 4. Hamana-Ko in total. 5. Northern half of Hamana-Ko. 6. Southern half of Hamana-Ko. 7. Mikawa Bay. 8. Nakano-umi. 9. Sukumo Bay. 10. Yatsushiro-kai. 11. Kyushu side of Yatsushiro-kai. 12. Amakusa side of Yatsushiro-kai. 13. Tokyo Bay. 14. Ogara-Numa. 15. Seto Inland Sea (1950). 16. Seto Inland Sea (1951). 17. Seto Inland Sea (1952). 18. Seto Inland Sea (1953). 19. Seto Inland Sea (1954). 20. Seto Inland Sea (1955).

of organisms present and also the production in weight of it differ somewhat. Anyhow the fact that S can be an index for the total weight of fisheries products should be considered to suggest that the suspended matter not retained by nets had a great significance to the productivity of the sea.

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A. 4.

The Estimation of Carbon in Phytoplankton

By D. H. Cushing

Steemann Nielsen's (1952) method of estimating the production of carbon as mg.C/m³./day has made it necessary for us to express the algal standing stock as mg.C/m³. There are two main methods of estimating phytoplankton:—

1. By counting and measuring the algae in a known volume of fixed sea water, using the sedimentation technique. From these estimates the volume in μ^3/litre, the algal volume, may be derived. There are two possible disadvantages to this method, firstly, that μ-flagellates are excluded and, secondly, that organic matter may be overestimated because of the inclusion of cell sap (Harvey, 1950).
2. By measurement of chlorophyll extracted from filtered sea water. There are two possible disadvantages, firstly, that the ratio of chlorophyll to organic matter varies and, secondly, that dead chlorophyll is included.

Any direct estimate of carbon is laborious and so a number of equivalents have been established. The basis of these equivalents is found in the work of Brandt and Raben (1919), which may be expressed in the following table:—

	Diatoms	Dinoflagellates	Copepods
$\frac{\text{Dried weight}}{\text{carbon}}$	5	3	2·2
$\frac{\text{Dry organic matter}}{\text{carbon}}$.	2	2	2

(Note: if carbon were all hexose sugars, the ratio of $\frac{\text{Dry organic matter}}{\text{carbon}} = 2{\cdot}5$; Sverdrup, Johnson and Fleming give 2·2).

Dried weight is defined as the weight of plankton from which the water has been driven off and which has not been ignited.

Dry organic matter is the difference between the dried weight and the ash. The wet weight of either phyto- or zooplankton is difficult to determine because of adherent water, but for *Chlorella*, Burlew (1953) gives

$$\frac{\text{wet weight}}{\text{dry weight}} = 4{\cdot}23;$$

for mixed phytoplankton, Grim (1939) gives

$$\frac{\text{wet weight}}{\text{dry weight}} = 5{\cdot}00.$$

The relationship between algal volume and carbon may be set up in two ways:

(i) $$\frac{\text{algal volume}}{\text{dried weight}} = 2{\cdot}54$$

(Riley, 1941; this estimate is a statistical one, which excludes the silica component in the algae from the chemical determinations but may include extraneous material, such as nauplii). Grim (1939), for diatoms gives 2·5, excluding silica.

$$\frac{\text{dried weight}}{\text{dry organic matter}} = 1{\cdot}57$$

(Riley, 1941; the estimate of dried weight is that given above. It will be noticed that Brandt and Raben find for diatoms, a factor of 2·5 and for dinoflagellates 1·5; their estimates are direct chemical ones and include the silica shells, etc.)

so $$\frac{\text{algal volume}}{\text{carbon}} = 8\text{—}10$$

(Cushing, 1957, used 2·5 to obtain dried weight from algal volume (Grim, 1939) and 2 to obtain dry organic matter (Brandt and Raben); he then reduced to carbon using 2·2 from Sverdrup *et al.*)

so $$\frac{\text{algal volume}}{\text{carbon}} = 11$$

(ii) 1 μg. chlorophyll = 34·5 μg. dried organic matter (Atkins and Parke, 1951, from Riley's Long Island measurements in 1941 and from Pace's measurements of a culture of *Nitszchia closterium* in

1939). 1 μg. chlorophyll = 0·139 mm³. algal volume (Gillbricht, 1952; this estimate is a statistical one, excluding the component of dead chlorophyll from the chemical determinations). Therefore 1 mm³. algal volume $= \frac{34.5}{0.139} = 248\,\mu$g. = 0·25 mg. dry organic matter.

So $$\frac{\text{algal volume}}{\text{carbon}} = 8$$

The relationship between chlorophyll and carbon may be set up in the following way:—

1 μg. chlorophyll = 0·139 mm³. (Gillbricht, 1952), dividing by 4 (2·54 × 1·57).

1 μg. chlorophyll = 34.8 μg. dry organic matter, which is very close to the estimate of Atkins and Parke given above.

So, 1 μg. chlorophyll = 17·3 μg. carbon (or 13·6 μg. if we were to use a $\frac{\text{dry organic matter}}{\text{carbon}}$ equivalent of 2.5).

Now, 1 P.P.U. (plant pigment unit) = 0·3 μg. chlorophyll (Atkins and Parke, 1951) or 0·227 μg. chlorophyll (Riley and Conover, 1956) or 0·26 μg. chlorophyll (Riley, 1941).

So,

1 P.P.U. = 3·9—5·2 μg. carbon.

These equivalents may be summarized in the following table:—

	Algal volume		Chlorophyll
Dried weight	2·54 (Riley)	2·5 (Grim)	
Dry organic matter	1·57 (Riley)	1·5—2·5 (Brandt & Raben)	$\frac{1}{34·5} = 0·029$
Carbon.....	2—2·5		2—2·5

$\frac{\text{algal volume}}{\text{carbon}} = 8$—10 $\qquad \frac{\text{chlorophyll}}{\text{carbon}} = 0·058$—0·074

Standard equivalents may be given:—

1 mm³. algal volume = 0·10—0·125 mg. C.
1 μg. chlorophyll = 13·6—17·3 μg. C.

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A. 5.

The Measurement of the Carbon Content of Diatoms, using the ^{14}C Technique: a Preliminary Note

By D. H. Cushing and H. F. Nicholson

The carbon content of diatoms in culture was measured in order to check the reliability of the algal volume/carbon ratio.

Let q be the weight of carbon produced in time t_1—t_0; let P_1 and P_0 be the number of algae at t_1 and t_0, respectively. Then the weight of carbon per cell is

$$q/P_1 - P_0.$$

Method

The culture used was *Skeletonema costatum* in sea water; the cultures were kept at constant light and temperature.

Experiments were run for 24 hours in a ^{14}C tank; the initial numbers of *S. costatum* were estimated from an aliquot with the sedimentation technique. The final numbers were estimated with the same method from the active culture, the sample of which was returned to the bottle when the count was complete.

The ^{14}C experiment was run at about the same light intensity and temperature as the cultures.

Results

The results may be shown briefly in Table 1.

Table 1. The weight of carbon in each cell of cultures of *Skeletonema costatum*

Bottle	Ampoules	P_0 n/ml.	P_1 n/ml.	$\frac{q}{P_1 - P_0}$
1	1	94,209	151,500	0·447 $\mu g.$ C$\times 10^{-4}$
3	2	48,736	146,182	0·323 $\mu g.$ C$\times 10^{-4}$
5	3	61,069	135,401	0·282 $\mu g.$ C$\times 10^{-4}$
9	3	81,159	133,361	0·614 $\mu g.$ C$\times 10^{-4}$
			Mean	0·42 $\mu g.$ C$\times 10^{-4}$

The average volume of *Skeletonema costatum* as measured was 324 μ^3 (n = 50); measured as a cylinder. It is assumed that the algal volume is equivalent to a wet weight; hence, the $\frac{\text{algal volume}}{\text{carbon}}$ ratio is $\frac{324}{42} = 7{\cdot}71$.

This is an estimate which excludes the weight of the silica shells of the diatoms.

B. 1.

Consumption of Nutrient Salts in the English Channel as a Means of Measuring Production

By L. H. N. Cooper

This method was used first in the western English Channel by Atkins (1923, 1930). Changes in phosphate were studied first and then supplemented by changes in nitrate, oxygen, and carbon which showed surprisingly good agreement (Cooper, 1933). Attention to detail, such as the salt error correction for phosphate, led to improved precision (Harvey, 1945, p. 144). The method has been much used. It was a very powerful and straightforward method for getting the order of magnitude of the minimum production in a given sea area.

We have reached a stage where we need to study the fine structure of events. Now that we have an overall mean or average picture, we need to study deviations from the mean, deviations which may arise from many causes. Unless many men or many ships can be brought to bear on the problem, it will become very difficult, at any rate in the English Channel, to understand this fine structure. Thus by 1939 it seemed to me that at Plymouth we had extracted everything we could from the method. Further advances with it were beyond our resources. My reasons were based on our own work in the 1930's but I can argue my case more surely from work done since the war.

The method depends on obtaining a firm estimate of the stock of phosphate or other nutrient at the time of the winter maximum and upon the assurance that the estimate represents a considerable area of the sea. As early as 1924 Atkins addressed himself to this problem and obtained a very satisfactory answer (Figure 1a). He surveyed a large area of the western English Channel and found the phosphate content very homogeneous, so much so that any attempt to draw contour lines through his figures would be silly. They are almost the same figure. In the following years he abundantly confirmed the uniformity of phosphate distribution in the western English Channel in winter. There seemed no doubt that the results at our station E 1, 22 miles from Plymouth, provided a base-line figure for the winter maximum of phosphate in the whole western English Channel. With the resources available he went to the limit to establish his case.

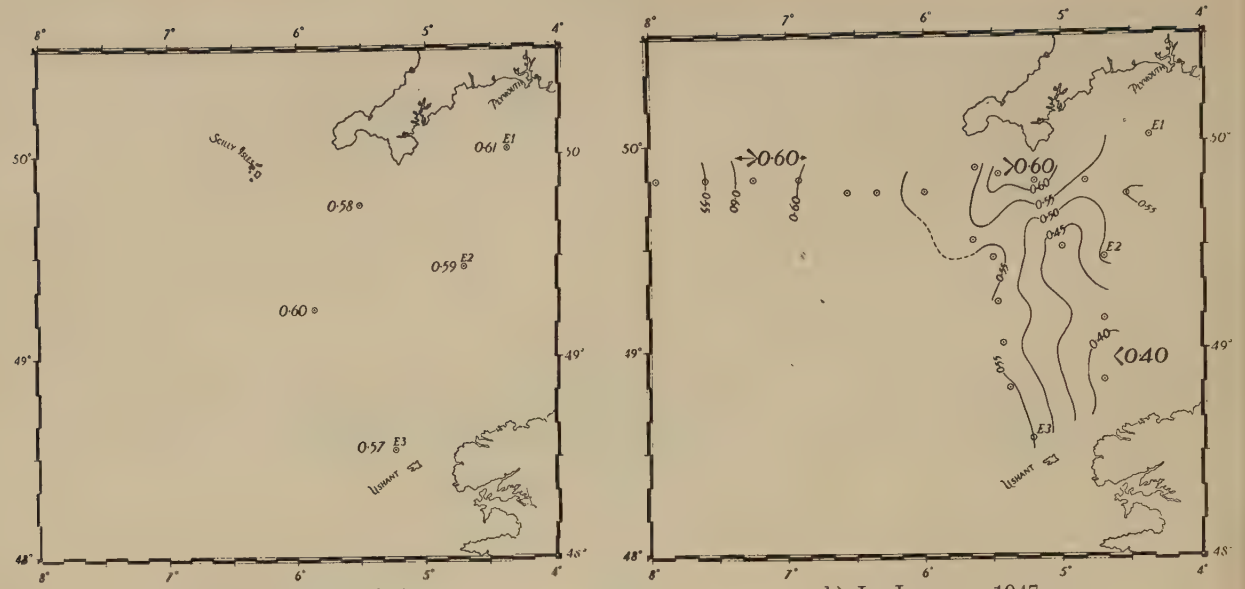
During the nineteen-thirties this result came under suspicion but our data were not sufficient for a firm conclusion.

The Midwinter Phosphate Maximum in January 1947

Consequently in January 1947 an intensive survey of the western English Channel (Figure 1b) was made. The time was chosen when phosphate (the integral mean of the water column) should be maximal and plant production minimal. The distribution of phosphate was very variable ranging from 0·37 μg.-atom/l. in the elongated patch of low phosphate lying near to the meridian 5° W. to 0·63 μg.-atom/l. along the parallel 49°50′ N. between 5°30′ and 7°15′ W., a ratio of 1·7:1. The mean content over the whole area was 0·53 μg.-atom/l. close to that found at E 1 (0·51 μg.-atom/l.), or as a mean from three of our standard stations, E 1, E 2, E 3 (0·52 μg.-atom/l.). Following a change of wind or current direction, either richer or poorer water might have arrived at any of the E series of stations.

In January 1947 it would have been very dangerous to assume that any single station was representative of anything. Moreover, the extensive pattern of phosphate-poor water was such that our standard network of stations would have completely missed it.

We worked the two metre stramin ring trawl (Y.F.T.) for standard half-hour hauls throughout the work. The richest hauls were at the stations with lowest phosphate (Table 1).



a) In February, 1924. b) In January, 1947.

Figure 1. Distribution in the western English Channel of phosphate-phosphorus (μg.-atom P/l.).

Table 1. Zooplankton at three mid-winter stations low in phosphate

(From information supplied by Mr. P. G. Corbin)

Station	A 4	A 5	X 4
Lat. °N.	49°09′	48°51′	49°30′
Long. °W.	4°42′	4°42′	5°00′
Date: 1947	7 Jan.	7 Jan.	22 Jan.
G.M.T.	0827–0857	1102–1134	0019–0053
Integral mean phosphate μg.-atom/l.	0·41	0·37	0·41
Zooplankton:—			
Sagitta elegans	3180	900	2020
S. setosa	220	80	330
S. serratodentata	—	—	1
Muggiaea atlantica	29	1	50
M. kochi	21	4	6
Aglantha	—	—	1
Themisto	180	5	420
Euchaeta hebes	1220	140	2020
Euphausian larvae	2	—	2020
Calanus	780	780	—
Rhincalanus nasutus	—	—	—
Mysids	7	—	190
Total young fish	nil	2	16

The indicator species are those we associate with the richly productive conditions of the 1920's when the stock of phosphate at the winter maximum was double that in this animal-rich area in 1947. It was much of a paradox that this phosphate-poor mid-winter water was so rich in animals. You may provide your own guesses as to the previous history of such water but there is every reason to believe that it would have continued productive; possibly more so than an exceptional water that we found around the Pembrokeshire coast in April 1952 with the very high phosphate content of 0·8 μg.-atom/l. but yet as barren biologically as any water we have ever sampled.

Water Exchanges in the Winter of 1950

The winter maximum of phosphate is usually found about the first fortnight of the year. On 18. January 1950 the phosphate content of the water column at station E 1 was 0·46 μg.-atom/l. If there should be an increase in phosphate during February, one always suspects a considerable water movement.

By 22. February there had in fact been an increase of 0·05 μg.-atom/l. at E 1 accompanied by a drop in temperature from 10·87° to 9·28° C. There are very frequent surface observations on the Lands End—Ushant line and it is clear that water colder than 10·1° never crossed it. There are also useful observations on 18. January from R.V. "Président-Théodore-Tissier" which showed that such water could not have arrived from the south-west, south, or south-east. It could have arrived only from the east. Such water was in fact present between the prime meridian and 2° W. (*Bulletin Hydrographique*, 1950, pp. 29—31) at 49°48′ N., 0°02′ E. (9·8°, 35·15 $^0/_{00}$ on 18. January), 50°21′ N., 1°52′ W. (9·2° and 35·21 $^0/_{00}$ on 10. February), 50°18′ N., 1°55′ W. (9·3° and 35·11 $^0/_{00}$ on 15. February). The water at E 1 on 22. February was part of a coastal current flowing westwards along the south coast of England from the Isle of Wight to Lands End and fed by water from the area between Beachy Head and Cap d'Antifer.

It was probably initiated by the strong gales which blew between 6. and 12. February. These pressed surface water eastwards to an extent which the Strait of Dover was unable to accommodate. The excess water could escape only towards the west. Studies of geopotential topographies show that such a return relief coastal current can be stable only if it is strongest near the bottom on the English side of

the English Channel. The increase in phosphate at E 1 between 18. January and 22. February 1950 can, therefore, be explained only by liberation of phosphate from the somewhat muddy deposits of Lyme Bay during the stormy period.

This water escaped around Lands End where the coldest water of the winter was found on 12. February:

49°52′ N., 05°56′ W. 10·4° C. 35·14 ‰ Sal.
50°04′ N., 05°53′ W. 10·1° C. 35·14 ‰ Sal.

There is very little doubt that the gales of mid-February 1950 resulted in a mass movement of water along the south coast of England and one result was an increase in the phosphate content at E 1 by 0·05 μg.-atom/l.

If there are no major water movements, there is little change in the temperature at E 1 in early March. However, in 1950 the temperature at E 1 rose from 9·28° on 22. February to 10·18° on 24. March. Salinity also increased by 0·14 ‰, i. e., the area around E 1 became occupied by more oceanic water from the south-west, the usual state of affairs.

For calculations of crop production from phosphate consumption we now have to choose between three figures, viz.:—

18. January	0·46 μg.-atom/l.
22. February	0·51 μg.-atom/l.
24. March	0·46 μg.-atom/l.

The highest figure represented a band of coastal water which was brought into being by a prolonged gale and then quickly disappeared round Lands End. The January figure represented a water mass which was twice replaced before the spring outburst could have started. The agreement between the January and March figures may be fortuitous.

The figure of 24. March is probably the one to take for a measure of the initial stock of phosphate. It is markedly lower than the apparent winter maximum on 22. February and would not have been selected by rule of thumb.

Partition of Nutrients

Again in the western English Channel we have a phenomenon which we do not fully understand. There is little doubt that our water is mostly renewed by an inflow of new water entering from the ocean west of Ushant. Before it reaches the neighbourhood of the Eddystone this water in most years loses one quarter of its phosphate content—about 0·15 μg.-atom/l.

The simplest explanation is that there is a process of partition. Our prevailing winds are south-westerly and westerly, pressing surface water up-Channel. Such water as cannot get through the Strait of Dover must escape as a bottom current down-Channel. Even when the water is not stratified, this will provide a mechanism for impoverishing the English Channel in nutrients. Plant production is confined to the shallow illuminated layers which tend to move up-Channel. Some of the herbivores are vertical migrants which may spend the night feeding in the upper productive waters which tend to move up-Channel and the day resting and defaecating in the deeper layers which may move down-Channel. Such a process would leave the Channel filled with impoverished water whilst enriched water too deep to be used again for plant production would escape to the west from our area of study.

It is not wrong to include phosphate which has suffered this fate in an estimate of productivity. Nonetheless we have the difficulty of the kind we have already discussed in deciding which phosphate concentration to take as our base-line—around 0·60 μg.-atom/l. which may be found in the southern Celtic Sea and adjacent Atlantic or around 0·45 μg.-atom/l. which is usual at E 1.

My remarks have been confined to the assessment of the winter maximum phosphate which gives the base-line for calculations of productivity. Similar uncertainty attends the assessment of the minimum figure. It is bedevilled by doubts as to how much a water mass has in common with the winter water sampled a few months earlier.

No more than a minimum assessment can ever be made by this method. Repeated usage of the same nutrient stock may well vary greatly from year to year and place to place. For the English Channel we already have the order of magnitude of this minimum crop production (Harvey, 1950, p. 106). We really need an assessment of total production but, beset by uncertainties I have described, I cannot see how to use apparent consumption of nutrients as a precision measure of even minimum production. In the English Channel we have exhausted the possibilities and should turn to other lines of attack.

Over the Fladen Grounds Mr. Steele has been attempting a parallel investigation. He is very well aware of the difficulties and has made a strong case that the method is of real value there. I would stress that my conclusions drawn for the English Channel are not a criticism of the exemplary work being done from Aberdeen in a quite different environment.

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B. 2.

Experimental Methods for Measuring Organic Production in the Sea

By E. Steemann Nielsen

Introduction

Theoretically it should be possible by means of experiments to investigate the production of organic matter by the phytoplankton in several ways: thus by measuring the uptake of CO_2 or N-compounds or by measuring the release of oxygen. In the sea, however, only two techniques have been used: a) the oxygen technique introduced by Gaarder and Gran (1927)—in USA called the light-and-dark bottle oxygen technique—and b) the carbon-14 technique introduced by Steemann Nielsen, 1952. Both techniques measure the rate of photosynthesis. The experiments are thus carried out identically with the exception of the way in which the rate of photosynthesis is measured, either by determining the oxygen production or by estimating the uptake of the radioactive tracer, ^{14}C.

I. The Oxygen Technique

The oxygen technique was introduced by Gaarder and Gran in 1927. In the best modification, water samples are collected from the various depths and siphoned into clear and black bottles. One or several of the bottles containing water from the different depths are used for measuring the concentration of oxygen before the start of the experiment. The others are again lowered to the depths from which the samples came. Winkler titrations are used for measuring oxygen.

In USA another modification of the oxygen technique has generally been employed, originally introduced by Riley (1939). Bottles are filled with surface water and suspended in a tub on deck for one or several days. This special technique has the advantage that many stations may be worked during a cruise. The likely production of air-bubbles (cf. below) is, however, a great disadvantage, one of several.

In all the modifications of the oxygen technique, bottles wrapped in black material are suspended simultaneously with the ordinary clear ones. In this way it is possible to determine both respiration and photosynthesis going on in the water samples. The oxygen content in the black bottle less that in the "initial" bottle represents the rate of respiration, whereas the oxygen content in the black bottle less that in the clear bottle represents the rate of photosynthesis. It must, however, be mentioned that respiration includes not only that of the autotrophic algae but also that of all other organisms, thus including that by bacteria and zooplankton.

The lower limit of the oxygen technique depends on the sensitivity of the Winkler technique. In a single titration it is impossible to determine oxygen with this technique better than $\pm$ 0.02 mg. O_2/l. In some coastal waters it is even difficult or impossible to determine O_2 with this accuracy. The Winkler determination is due to the oxydation of manganous hydroxide to manganic hydroxide by means of the dissolved free oxygen. An equivalent amount of iodine is later developed which is titrated by a solution of sodium thiosulphate in the presence of starch.

The change in colour of the starch may prove difficult, as the blue colour may appear again after the first disappearance. All single titrations have to be made at exactly the same speed and the starch must be added as late as possible as the colour shift otherwise becomes questionable. In extremely plankton-rich water the applicance of the Winkler technique may be absolutely impossible as the iodine is taken up by the fatty oil in the plankton (cf. Steemann Nielsen and Aabye Jensen, 1957, p. 76).

In clear oceanic water the accuracy of Winkler titrations is by far the best. As to be shown below, the technique is here unfortunately of little use for measuring the rate of photosynthesis by plankton

algae as the rate of photosynthesis, on the one hand, is much too low to allow experiments of short duration and, on the other hand, severe complications arise in experiments of longer duration due to the development of a heavy bacterial growth when sea water is enclosed in bottles (see below).

In normally productive coastal water the oxygen technique for measuring organic productivity is applicable in most cases, but many pitfalls may turn up. Duplicates or, still better, triplicates of all the different bottles must be used. The single bottles must furthermore be analysed in a way allowing duplicate and triplicate analyses of oxygen. This may be done by using a semi-micro technique allowing the titration of, for example, 5 ml. portions of the samples. Accidental failures in titration may thus be avoided.

Another important caution to be taken is to discard all bottles in which air bubbles have appeared. In very productive water it is of course possible to get not too bad values from such bottles with air-bubbles, if these are relatively small. In less productive water the influence of air bubbles is in all cases too serious. Duplicates vary then in most cases considerably presumably owing to different sizes of the bubbles. The table presented by Gessner, 1944, p. 729, shows quite clearly how much the oxygen content may vary in duplicates and triplicates of light and dark bottles, making any evaluation of the differences between the two kinds of bottles impossible.

It is possible in several ways to avoid the production of air bubbles in the experimental bottles. The easiest way is to place the bottles somewhat below the surface. Already at a depth of 1 m. the pressure is increased by 10 %. The author has never seen any production of air bubbles at that depth. At 30 cm. below the surface, however, air bubbles may be produced. The production of air bubbles is often due to the fact that the temperature rises during the course of the experiment. Water is able to dissolve larger quantities of the gases at a low temperature.

The production of air bubbles can be avoided also in another way, first of all to be used in laboratory experiments where the bottles usually cannot be placed below a sufficiently deep water layer. Before the start of the experiment the experimental water is mixed with water with a low gas content. Such water may be produced in several ways, for example by boiling and cooling without contact with the atmosphere, or by pumping the air out.

By paying attention to all the weak points in the oxygen technique it is possible to use it if the rate of photosynthesis per volume is sufficiently high. It is, however, easy to understand why so many workers after a first unsuccessful attempt to use the technique have abandoned it again. If to be used properly, the oxygen technique demands a good deal of training and experience.

Riley (1941) has tried to evade the difficulties in using the oxygen technique by making a considerable number of single observations in an area and by evaluating the monthly averages for the whole area.

Measurements of organic production by the oxygen technique in plankton-poor water

In very plankton-poor water a special difficulty appears. The difficulty is not especially due to the oxygen technique. If sea water is enclosed in bottles, bacteria start at once to grow in great quantities. Due to the solid surfaces now present, organic matter is at once attacked by the bacteria. It is present in concentrations of about 1·2—2·0 mg. C per litre in

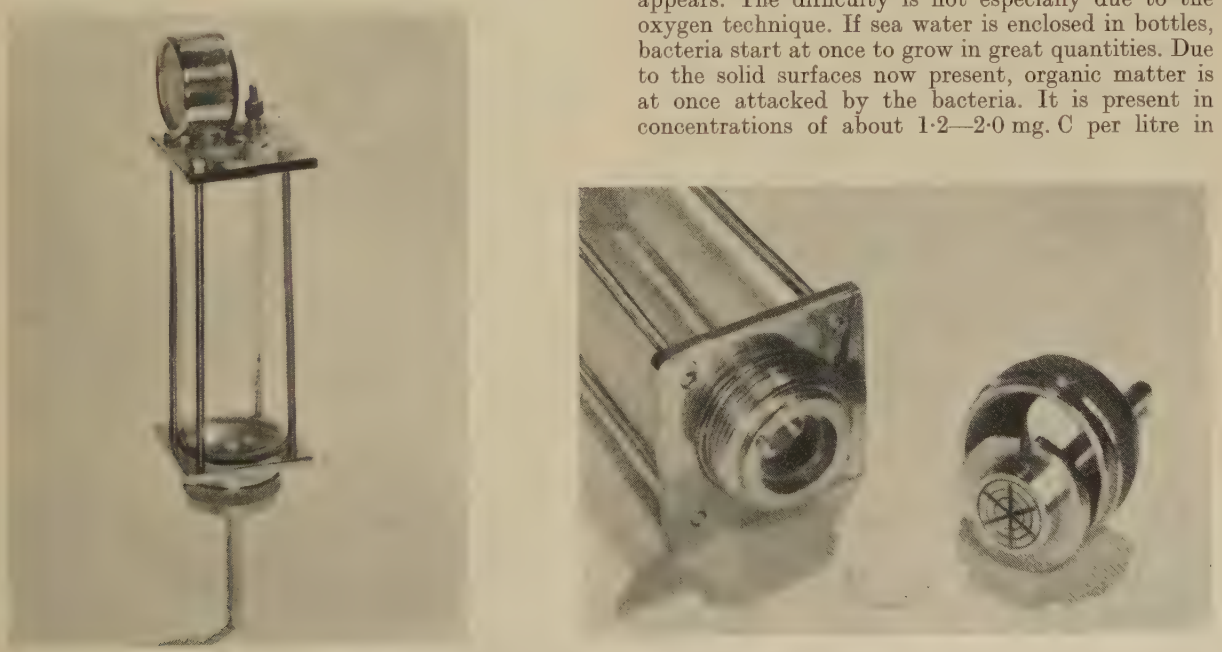

Figure 1. An apparatus for filtering plankton by pressure.

all sea water, but cannot apparently be attacked when the water is *in situ* because of the lack of solid surfaces—cf. for example ZOBELL (1936). The rate of respiration by these bacteria is often as high as about 0·2 mg. O_2/l. per day. The bacterial growth seems to start earlier when the concentration of plankton is very low.

According to experiments of another kind and to theoretical considerations the rate of photosynthesis at the surface is of the order of magnitude 0·003 mg. O_2/l. per day in the very oligotrophic tropical parts of the oceans—cf. STEEMANN NIELSEN (1954). Measurements here by the light-and-dark bottle oxygen technique often show a difference between the light and dark bottles of up to 0·17 mg. O_2 per litre per day (RILEY, 1939; RYTHER, 1955). RILEY's and RYTHER's explanation that the differences between the light-and-dark bottles in such waters should be due to photosynthesis can not be agreed to. STEEMANN NIELSEN (1952, 1954, 1957) has given several separately sufficient reasons for the rejection of the explanation.

As an example we may take measurements from the Sargasso Sea from June 1954; cf. RYTHER (1954), Table 1. These measurements were made by using triplicates of both light-and-dark bottles. The precaution was taken that no air bubbles were produced. The difference—presented as rate of photosynthesis—70 mg. C per m^3. per day at the surface is statistically significant. The water was—according to a personal communication by the observer Mr. DON FORSTER—deep blue as always in the Sargasso Sea. According to these measurements, the rate of photosynthesis in the whole photic layer (about 120 m. deep) would be at least 4 g. C/m^2. per day. If correct, this would be the highest rate ever found in a natural habitat of plankton algae!

Plankton algae producing 70 mg. C/m^3./day at the surface have at least a content of 70 mg. C, corresponding to an algal volume of 700 mm^3./m^3. According to LOHMANN's and HENTSCHEL's determinations, revised (multiplied by a factor three) by STEEMANN NIELSEN and AABYE JENSEN (1957) this volume is about 10 mm^3./m^3. in the Sargasso Sea and in the corresponding area of the South Atlantic. A production of 0·7 mg. C/day/m^3., as measured by RYTHER with the carbon-14 technique, fits excellently.

In some way or other light must be able to reduce the bacterial activity in the bottles containing oligotrophic ocean water. Based on the results of some experiments, STEEMANN NIELSEN (1955b) has ascribed this reduction as due to the production of antibiotics by plankton algae. Recent experiments in the author's laboratory using filtrates from plankton cultures have corroborated the view. All species of plankton algae investigated seems to excrete a substance which then is transformed into another substance adsorbing to surfaces. In this state it is active against bacteria. Growth promoting factors are produced at the same time.

Special techniques are needed both for obtaining active filtrates from plankton cultures and for measuring the activity of the filtrates.

In plankton-rich water the production of antibiotics is very likely to be so considerable even in the dark, that there is practically no difference between the development of bacteria in the light and in the dark. It is thus possible to explain why RYTHER (1956) was unable to observe any difference between his illuminated and his dark cultures.

It is in the same way easy now to understand why in the experiments with water from the Sargasso Sea—partly presented by STEEMANN NIELSEN (1954), Table 3—both the rate of O_2 production and the rate of O_2 consumption was 2—4 times higher in paper-filtered water than in non-filtered water. According to the simultaneous measurements with the tracer technique the rate of photosynthesis was reduced to about one quarter by filtering the water through filter paper. The duration of the tracer experiments was six hours.

Table 1. O_2 experiments with surface water from the Sargasso Sea, 3.—6. June 1952

Triplicates used, each titrated twice, mg. O_2/1. ± 0.01

Water	Initial bottles	Clear Bottles	Dark bottles
Non-filtered	6·60	6·57	6·51
Paper-filtered ...	6·59	6·61	6·37

"O_2 consumption" per litre in three days:—
non-filtered 0·09 mg.
paper-filtered 0·22 mg.
"O_2 production" per litre in three days:—
non-filtered 0·06 mg.
paper-filtered 0·24 mg.

Using water filtered through paper the rate of "photosynthesis" according to the oxygen technique was about 200 times higher than the rate according to the carbon-14 technique. Using non-filtered water it was only 15 times higher.

II. The Carbon-14 Technique

For the first time this technique was used during the cruise of the "Galathea" round the world 1950—1952. It was described by STEEMANN NIELSEN (1952). A more detailed survey of the technique, first of all concerning its use on the "Galathea" expedition is presented by STEEMANN NIELSEN and AABYE JENSEN (1957).

In the ^{14}C method the incorporation of the tracer in the organic matter of the plankton algae is used as a measure of the production. A definite amount of $^{14}CO_2$ is added to the sea water, the productivity of which is to be measured. The content of CO_2 (total) in this water must be determined or estimated. If we assume that $^{14}CO_2$ is assimilated by the plankton

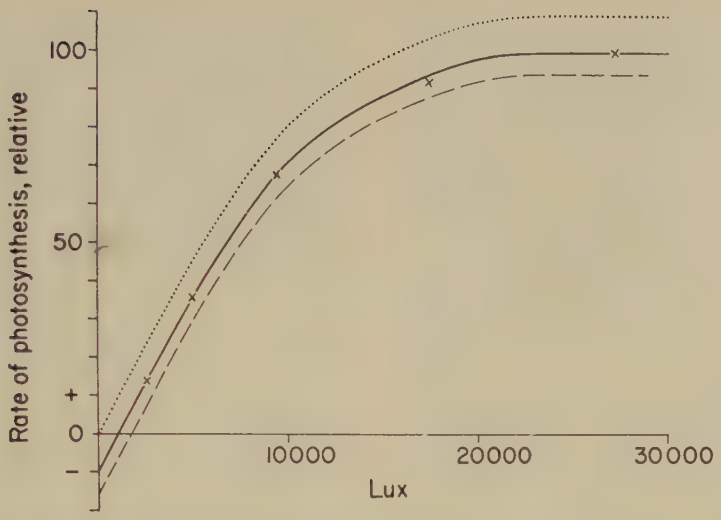

Figure 2. Light intensity and rate of Photosynthesis in the Tasman Sea.
Dashed line = net production, dotted line = gross production.

algae only through photosynthesis and that $^{14}CO_2$ is assimilated photosynthetically at the same rate as $^{12}CO_2$ by determining the content of ^{14}C in the plankton after the experiment, we also determine the total amount of carbon assimilated. It is only necessary to multiply the amount of ^{14}C found by a factor corresponding to the ratio between CO_2 (total) and $^{14}CO_2$ in the water at the beginning of the experiment. The amount of ^{14}C assimilated is determined by measuring the β-radiation from the plankton, which is retained by collodion filter.

As ^{14}C has a half-life period of more than 5,000 years, samples with a definite content of $^{14}CO_2$ can be kept in stock. 1 ml. samples of water are used, which has nearly the same content of total CO_2 as normal sea water. The water has a content of NaCl a little lower than oceanic sea water. The ^{14}C samples are kept in sealed glass containers (ampoules). They are autoclaved immedately after preparation as otherwise the possibility of bacterial growth and associated dark fixation of the tracer may cause trouble. The water used for preparing the $^{14}CO_2$ solution has to be distilled immediately before using as otherwise the bacteria count may be too high.

As the small ampoules are extremely difficult to clean on account of their narrow necks, previously sealed ampoules of Jena fiolax glass are used. These are not only absolutely clean inside but are sterile, due to sealing during their manufacture. The ampoules are opened just before being filled.

Whereas the filling of the ampoules may be done appropriately without difficulty the subsequent sealing may prove difficult. A special burner for this process is recommended (cf. Steemann Nielsen and Aabye Jensen, 1957, Figure 2). Workers not especially acquainted with pharmaceutical technique are recommended to autoclave the filled and sealed glass containers in a solution of methylene blue (0·1 %). On cooling, the blue solution is sucked into any glass containers which have not been effectively sealed. These are thus easily recognizable.

The $^{14}CO_2$ solution in the ampoules must have no contamination of any kind.

^{14}C is supplied in the form of $BaCO_3$ with about 5 per cent. of the carbon in the form of ^{14}C. As $BaCO_3$ is insoluble the CO_2 is converted into Na_2CO_3. A specially constructed vacuum line is used (cf. Steemann Nielsen, 1952, p. 121).

For normal work 1 ml. ampoules of four different strengths are prepared. The different containers are made in series of 200, and are each wrapped in appropriately coloured tissue paper. Within series of the same calculated strength the correspondence is fairly close. In later calculations values for the particular series is always used. The Danish series of the greatest strength contain 8 μC per ampoule, the series of the weakest strength, 0·2 μC.

It is possible commercially to buy solutions of $NaH^{14}CO_3$. The manufacture of the ampoules is thus easier as no distillation of the CO_2 has to be done. It is, however, to be recommended to start from $BaCO_3$. Only in this way is it possible to control in an effective way the preparation of the ampoules with respect to contamination.

For counting the radioactivity carbon dioxide is precipitated as barium carbonate, which is then filtered off by the usual collodion filter (see below) or better by a millipore filter (Lowel Co., USA) which does not change weight if an aqueous solution is passed through. The area must be the same as that used when counting plankton.

Regarding the preparation of barium carbonate samples and the determination of a self-absorption curve necessary for extrapolating the measurements

of the $BaCO_3$ samples to weight zero, the reader is referred to CALVIN *et al.* (1949), e. g., p. 31 and p. 104, f.f. By adding carrier the weight of the $BaCO_3$ samples is put at about 20 mg. per 3 cm^2.

A special self-absorption curve must be made for each Geiger tube. The curves are not strictly exponential! With end-window Geiger tubes of the same type, and with the same mass thickness of the windows, practically identical curves are obtained.

Some technical skill is necessary to make an adequate self-absorption curve. It presupposes further familiarity with tracer work when preparing ^{14}C ampoules and measuring radioactivity. On the other hand, the practical application of the carbon-14 technique in the field does not presuppose any expert knowledge. The author has therefore asked UNESCO about the establishment of an international centre from which ^{14}C ampoules ready for use could be distributed and where the filters with the plankton later could be counted for β-radiation; cf. p. 14 of this volume.

The determination of the photosynthetic activity of plankton algae by the carbon-14 technique

After the contents of a ^{14}C ampoule has been added to a water sample containing plankton algae and the sample has been exposed to light, part of the ^{14}C is found in the organic matter of the plankton algae. If this quantity of ^{14}C is to be used as a measure of the photosynthetic intensity, we must know both the content of total CO_2 in the water and the ratio

$$\frac{^{12}C + ^{13}C + ^{14}C}{^{14}C}$$

in the total CO_2 of water. As a rule, however, we shall avoid using absolute amounts of ^{14}C, as only the activity of the experimental water and the activity of the plankton algae are to be determined. If, under identical conditions, the activity of the experimental water is a, that of the plankton algae b, and the content of total CO_2 in the water c, then the total amount of CO_2 assimilated by the algae is $\frac{b \times c}{a}$.

This is on the assumption that $^{14}CO_2$ is assimilated at the same rate as $^{12}CO_2$ and that ^{14}C is not incorporated in the algae in any other way than through photosynthesis. This question will be discussed in detail below.

If pH, titration alkalinity, and temperature are known, it is possible, according to BUCH (1945), to determine total CO_2 in fresh water provided that the titration alkalinity does not approach zero. In sea water it is also necessary to know the chloride content, which has partly a direct effect, partly an indirect effect through the boric acid, the concentration of which is proportional to the chloride concentration. In oceanic water the calculation of total CO_2 thus becomes slightly more complicated than in fresh water (see HARVEY, 1945, pp. 52—73).

If Cl $^0/_{00}$ of the sea water is known, it is not necessary to determine the titration alkalinity A, as this is equal to 0·000123 Cl $^0/_{00}$ expressed in equiv./l. With brackish water, as with fresh water, it is necessary to determine the titration alkalinity.

As pH varies so little in oceanic surface water, it is unnecessary to determine pH unless very high accuracy is aimed at. If it is put at 8·20, the error in total CO_2 will be 2 to 3 $^0/_0$ at the most. Normally for the present purposes it will be sufficient to put total CO_2 at 90 mg./l. for normal oceanic surface water.

After an experiment the plankton algae must be filtered off by a smooth collodion filter with sufficiently narrow pores (the maximum pore width must be about 0·8 μ). The membrane filter: grob, Stufe 2, from Membranefiltergesellschaft, Göttingen, or millipore filters from Lowel Co., USA, may both be recommended. The latter are the fastest but more breakable. For use by non-scientists the first ones must be preferred. Filtering may either be done by suction (cf. CALVIN *et al.*, 1949, Fig. 40) or by pressure. Figure 1 shows a device used now for several years in Denmark. The pressure is produced by an ordinary bicycle pump. Several slightly different models have been in use. Dr. VOLLENWEIDER, Alexandria, has contributed to the design of the final model.

The membrane filters with the plankton must be placed in special holders for drying as they otherwise will shrink. This is not necessary when using millipore filters. Afterwards they are placed for 20 minutes in a closed container above fuming hydrochloric acid. This is done in order to remove carbonate to be found, e.g., in coccoliths or in the filter. No other rinsing of the filter is then necessary. Immediately afterwards the filters must be dried in a desiccator—also containing some soda lime—and if they are not examined at once, kept there.

Any window-less or end-window Geiger-Müller counter tube with a very thin window may be used. The author prefers an end-window tube with a window radius of only 7·5 mm. The background can thus be kept very low.

If the geometry used in measuring the barium carbonate for determining the activity of the contents of the ampoules is identical with the geometry used in measuring the activity of the plankton, the two activities may be directly compared. If the weight of plankton is below 0·1 mg. per 3 cm^2., self-absorption in the plankton may be disregarded.

At the start of an experiment the content of an ampoule is added, by means of a glass pipette, to the bottle containing the sample in which the photosynthesis is to be measured. The container is rinsed with a little water from the sample.

A sample of elementary carbon containing ^{14}C, mounted on the same mounting ring and base as normal samples and with the same area is used as a standard.

If the amount of organically bound ^{14}C in the plankton after an experiment is to give an absolute measure of the intensity of photosynthesis (the gross

production) the following conditions must be present: (1) No $^{14}CO_2$ must be incorporated in organic compounds except through photosynthesis, (2) the rate of assimilation of $^{14}CO_2$ must be the same as that of $^{12}CO_2$, (3) no $^{14}CO_2$ must be lost by the respiration which takes place simultaneously with photosynthesis, and (4) no organic matter must be lost by excretion.

None of these conditions are, however, quite fulfilled. Although the importance of each of them is only relatively slight at high light intensities and one of them has the opposite effect to the others it is necessary to introduce a correction (valid at high light intensities) covering them all.

Dark fixation in plankton algae is mostly about 1—2 per cent. of the fixation at optimal light intensity. In polluted waters where huge quantities of bacteria are found, it may be of extraordinary importance.

$^{14}CO_2$ is photosynthesized about 5 per cent. slower than $^{12}CO_2$; (cf. ANDERSON and LIBBY, 1951, and STEEMANN NIELSEN, 1955a). In light about 50—70 per cent. of the CO_2 produced—or expected to be produced—by the respiration is in some way or other identical with CO_2 fixed during photosynthesis as experimentally shown by STEEMANN NIELSEN (1955a). This does only concern experiments of relatively short duration. In ^{14}C experiments of very long duration practically all CO_2 respired will be of the same kind as that assimilated. We now have static conditions. The carbon-14 technique, therefore, measures net production in such experiments.

If the rate of respiration is 10 % of the photosynthetic rate—the normal proportion found at optimum light in nature—the interaction of respiration and photosynthesis will have the effect in short time experiments that the rate of gross production is about 6 % higher than the rate directly measured by the carbon-14 technique.

If these three corrections are added together we get a total correction of $(+5 + 6 - 1) = +10$ %. The correction + 10 % is only valid if the rate of respiration is 10 % of the rate of photosynthesis.

If the rate of respiration is 5 % of the rate of photosynthesis, the correction should only be 7 %. If, on the other hand, the rate of respiration is 25 % or 50 % of the rate of photosynthesis, the correction should be about + 20 % and + 50 %, respectively. Therefore, it must be stated that the ^{14}C technique is only applicable with reservations if the rate of photosynthesis is not considerably higher than the rate of respiration.

By the author and his collaborators nearly 100 curves, showing the dependence of photosynthetic rate on light intensity, have now been made with water from the photic zone in both oligotrophic and eutrophic areas from all temperature provinces. It is possible to extrapolate the rate of respiration from these curves. In sub-surface water the ratio of rate of respiration to rate of optimal photosynthesis is less than about $^1/_{10}$. The highest ratio found in surface water is $^1/_4$. Normally the ratio is about $^1/_{10}$ also here. In tropical-oligotrophic surface water only small ratios have been met with. Figure 2 shows an ordinary light assimilation experiment. The measured rate is corrected both to show net and gross production. The latter curve shows also the rate of respiration.

The *in situ* experiments are preferably made only during the daytime, thus decreasing the influence of respiration by the factor 2. Finally, these measurements are best if not carried on right down to the supposed compensation depth. Special corrections can be made for the measurements from the lowest part of the photosynthetic layer. It should however, be mentioned that even considerable deviations in the photosynthetic rates in the lowest part of the photosynthetic layer only slightly influence the magnitude of organic production per unit surface.

No correction is made for organic material excreted by the algae. In the light of the modern knowledge concerning algal physiology there is no reason to consider such an excretion as being of any pronounced importance in experiments of short duration at normal light intensities (cf. MYERS and JOHNSTON, 1949; FOGG, this volume, p. 57).

A duration of "tank" experiments—cf. below—of about 4 hours may be recommended. If the duration of an experiment with surface water taken in sunshine is very short, the after-effect of light inhibition may be too pronounced. A long duration of the experiments is, on the other hand, to be avoided, especially when using oceanic plankton which is not able to stand the conditions in experimental bottles for a long period. As even bacteria may develop in huge quantities fixing $^{14}CO_2$, the technique may be completely unfit for any measurement of photosynthesis.

RYTHER has in a series of articles (cf., f. example RYTHER, 1954) expressed his view that in oligotrophic-tropical water the rate of photosynthesis nearly equals the optimal rate of photosynthesis. In this way he tried to explain the discrepancy between the results obtained by the oxygen technique and the carbon-14 technique in such water. However, STEEMANN NIELSEN and AL KHOLY (1956) has shown the impossibility of this explanation.

The *in situ* method and the "tank" method

For determination of the production of matter under 1 m². of surface, a method resembling GAARDER and GRAN's oxygen method can be used. Instead of measuring oxygen metabolism, the intensity of photosynthesis is determined by the ^{14}C method.

With a non-metallic water bottle of some kind water is taken from the various layers of water in which photosynthesis is believed to take place. This may be decided by measuring the penetration of light into the sea. After a bottle (e. g., 100 ml. Jena-bottle with glass stopper) has been filled and the content of a ^{14}C ampoule introduced, the bottle is lowered to the depth from which the sample came. This is done either immediately before sunrise or when the sun is at its meridian altitude. The bottle is raised

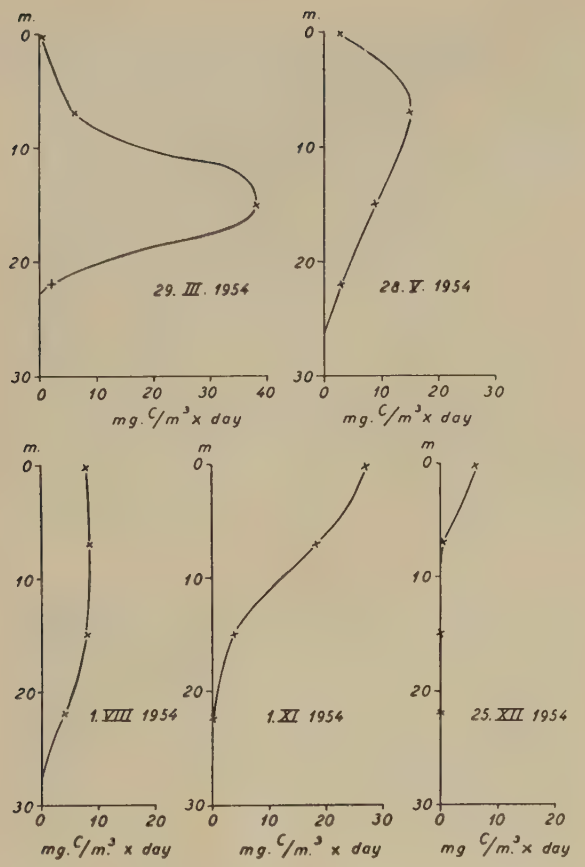

Figure 3. Normal *in situ* experiments in the Kattegat. The light conditions were in all cases favourable.

again, either in the first case when the sun is at its meridian altitude, or in the second case immediately after sunset. In both cases, the photosynthesis measured relates to half a day, and the value found has only to be multiplied by 2 to relate to the whole day. To estimate the total photosynthesis below 1 m². surface of the sea, the determinations from the single depth are presented graphically, see Figure 3. The area to the left of the curve represents the photosynthesis below the surface.

The *in situ* method is not always applicable on expeditions. It is much too time-consuming. It has, however, now been used regularly for several years both in Greenland and Danish waters. Lightships are particularly suitable for such studies.

If the rate of respiration is put at 5—10 per cent. of the maximum rate of photosynthesis found, the compensation point during the day should be found at the depths where 5—10 per cent. of the maximum photosynthetic rate is measured. The compensation point for 24 hours—a day and a night—is thus found at the depth at which 10—20 per cent. of the maximum photosynthetic rate is measured. This depth corresponds in most cases approximately to the depth at which one per cent. of the surface light (blue + green) is found. Because of the intermixing of photosynthesis and respiration the compensation depth can only be presented with some reservation. This is, however, only of little importance for the computation of the rate of photosynthesis per surface unit.

With the "tank" method, water samples from the various depths, after addition of ^{14}C, are exposed to a definite light intensity in a water-bath at the same temperature as the sea. After 3 to 4 hours the plankton is filtered off and the photosynthetic intensity determined in the usual way. From the value of the photosynthetic intensity found for a given light intensity and temperature, the production of matter by photosynthesis through 24 hours can be calculated if we know, firstly, the dependence of photosynthesis on the light intensity and, secondly, the light intensity at various times of the day at the depths from which the samples originate.

In practice, however, such a procedure would mostly be too much time-consuming. On the "Galathea" expedition another procedure was used[1]). At five tropical and subtropical stations measurements were made at the same time both with the *in situ* method and the "tank" method. All series showed practically the same relation between the results obtained with the two methods. It was thus possible to derive a formula for organic productivity based on measurements by the "tank" method.

Production of matter per m². per 24 hours =

$$\frac{(2a + 2b + c) \cdot d \cdot e}{5 \cdot 2} \text{ mg. C}$$

where

a is the photosynthesis in mg. C/hour/m³. at 18,000 lux in surface water;
b is that in water from a depth with 10 per cent. of the surface light;
c that in water from a depth with 1 per cent. of the surface light;
d is the depth in m. at which the light is 1 per cent. of the total quantity of blue and green light at the surface, and
e is the number of hours from sunrise to sunset.

As the photosynthesis taking place in the deepest layers of the photosynthetic zone is of less quantitative importance than that taking place in the upper and middle layer, the value for photosynthesis at 18,000

[1]) Ryther (1956 b) described a somewhat similar method for estimating daily photosynthesis beneath a surface area = P, from measurements of (1) photosynthesis of a sample of the population at light saturation = $P_{sat.}$ (2) the extinction coefficient of light in the water = k, and (3) the daily surface radiation. He presents the formula $P = \frac{R_s}{k} \times P_{sat.}$ where R_s = total relative photosynthesis within the euphotic zone for the appropriate value for total daily surface radiation. Practically the same results are obtained by employing this method or the method used on the "Galathea".

lux in the water from the depth with 1 per cent. of the light is entered in the formula at half its value.

In tropical—sub-tropical surface water a photosynthetic rate of about 70 % of the maximum rate is found at 18,000 lux with the special light source used on the "Galathea" expedition (tubular fluorescent lamps, Philips 33a).

The values directly obtained by the formula given above correspond to absolutely bright weather. If 10 per cent. is subtracted, average values for "normal" weather are obtained in most tropical—sub-tropical areas.

A rather similar formula may be derived for water from higher latitudes during summer. If the "tank" method has to be used it would be wise to construct the formula according to experience. If a formula given by another worker is used, it is necessary to use exactly the same light source. Otherwise, the statement of the light intensity in lux is rather meaningless.

As a pronounced light depression of photosynthesis is found near the surface in bright weather it is important to keep the duration of "tank" experiments with surface water relatively long (4 hours). It is also recommended in bright weather not to start "tank" experiments in the late afternoon, when the light depression at the surface is highest.

Comparison between results obtained by the carbon-14 technique and by other techniques

When comparing results obtained with the oxygen technique and the carbon-14 technique *inter se* it must be remembered that in photosynthesis of natural plankton the CO_2/O_2 exchange quotient is about 0·70—0·75 (cf. Myers and Johnston, 1949). If the photosynthetic rate can be measured with a sufficient accuracy using the oxygen technique, an extremely good agreement is found between the two techniques. The experimental time must be kept so short that no complications arise (cf. Steemann Nielsen, 1952, 1957; Ryther, 1954).

The ratio between gross production and net production

The maximum rate of photosynthesis in phytoplankton collected directly from the sea is mostly about 10 (5—20) times the rate of respiration. If the ratio is put at 10, the daily rate of respiration by the phytoplankton in the photic layer below a surface unit is 40 per cent. of the daily rate of photosynthesis below the same surface unit. This is at least correct for the tropics and sub-tropics during relatively clear days. The net production is thus 60 per cent. of the gross production (cf. Steemann Nielsen and Aabye Jensen, 1957). On account of the longer days at higher latitudes during summer, net production is decisively more than 60 per cent. of gross production on clear days. On dark days it is quite the opposite. During mid-winter even in the vertically extremely stratified Danish waters a net production is not found at all on dark days. Ryther (1956b) has shown the same for the waters near Rhode Island.

During the cruises of the "Dana" in the North Atlantic and arctic waters in 1956 and 1957 the dependence of the photosynthetic rate on light intensity was measured of the plankton from 3 depths on altogether 35 stations. An approximate rate of respiration could thus be determined by extrapolation — cf. Figure 2. This mode of measuring the rate of phytoplankton respiration must, however, be considered too laborious to be used as a standard technique. Ordinarily, we must be content with estimating it.

An imitated *in situ* method

The *in situ* method is as already mentioned time-consuming and therefore mostly too expensive to be used on expeditions with big ships. The "tank" method, on the other hand, requires preferably the assistance of a person familiar with photosynthesis. It would therefore be of considerable importance to have a method resembling the *in situ* method but to be used when the ship is moving.

The simplest way is to take surface samples only and, after introduction of ^{14}C, suspend them in a tub on deck such as done by Riley (1939), using the oxygen method. As a supplement to the experiments in the tank, such measurements have been made during the recent cruises of the "Dana" in arctic waters. As the standing crop of plankton often varies considerably with depth it is, however, recommended to take samples from several depths of the photic layer. In order to imitate the light conditions in the depth, plates of neutral glasses are placed above the bottles with sub-surface water. We have used neutral filters with the same rate of absorption in the whole part of the spectrum where photosynthesis takes place. As in the sea the light quality changes with depth the imitation is only approximate. Figure 4 presents

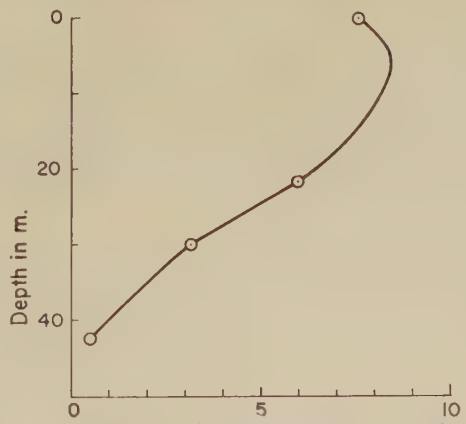

Figure 4. An imitated *in situ* experiment in the North Atlantic. (52°00′N. 32°42′W.) 16th August 1955.

an imitated *in situ* curve from such measurements in the North Atlantic during a sunny day in August 1955.

By combining colour filter glasses and neutral filter glasses it should be possible to imitate the light conditions in a much better way. Perhaps such a technique should be recommended as a standard method for use on cruises of research vessels.

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B. 3.

Selected Features of the Isotopic Carbon Primary Productivity Technique[1])

By Maxwell S. Doty and Mikihiko Oguri

I. Introduction

Steemann Nielsen first outlined (1951) and employed (1952a & b; 1954, 1957 (with Aabye Jensen)) this method on the deep-sea expedition of the "Galathea". Subsequent to that time Steemann Nielsen (1955a; 1956 (with Al Kholy)); Riley (1953); Ryther (1956a & b); Ryther & Vaccaro (1954); and Vaccaro & Ryther (1954) have published various pertinent comments. The method has proven useful and has been applied to various problems of phytoplankton productivity, in addition to the above, by Brouardel & Rinck (1956); Doty, *et al.* (1955, 1956a & b, 1957); Guillard, *et al.* (1955); King, *et al.* (1957); Miyake (1954); Steemann Nielsen (1955a & b); and Yentsch & Ryther (1957).

This technique has been developed further in the Botany Department of the University of Hawaii to where it is routinely included on oceanographic data-gathering voyages. The development of the technique has been described variously at meetings and these descriptions distributed as mimeographed reports (Doty, 1954, 1955, 1956). Some of the important manipulatory and reliability features are discussed below.

2. Collection

A comparison of collecting methods (Table 1) has shown water dipped in a plastic bucket to give the highest productivity measurements. Articles made of metal should not contact the sample water, since contact with metal and other materials has been found to greatly reduce carbon fixation. Table 2 illustrates comparatively the results of contact with a number of substances.

Collection should be made, as much as possible, away from parts of the vessel from which there is any discharge (e.g., from bilge, etc).

In every case three uniform glass-stoppered, pyrex bottles are filled. Two bottles ("light" bottles) are of clear glass. The third bottle (the "dark" bottle) is made from a stock of the same clear glass bottles used

Table 1

A comparison of sampling methods and sources, with the results calculated in per cent. of standard method value. The collecting of water by the standard method was done at the same time and place as each other entry

Plastic bucket and funnel (standard method)	100	Nansen bottle water	31·4
Pumped through plastic hose and iron centrifugal pump at Salpa station 2	8·9	Nansen bottle water (a different bottle)	34
at Salpa station 3 ..	8·3	Snatch-bottle sampler	107
at Salpa station 4 ..	5·4	Van Dorn sampler	100
Pumped as above, light	6·5	Pyrex flask of distilled water replaced by sea water sample by aspiration	
Pumped as above, light	8·5	light	96 to 103
Pumped as above, dark	75·9	dark[1])	70·3

[1]) % of standard method dark bottle sample value.

Table 2

Materials in sample bottles during incubation as small chunks in contact with water. Results in per cent. of standard method values

Plastic bucket and funnel (standard method)	100	Tygon	49·6
Hose material, plastic	49·5	Lucite	108
Plywood	38·7	Lucite	97·6
Neoprene	25·7	Plexiglass	97·6
		Paraffin	95·2

[1]) This technique was developed with financial and logistic assistance provided on contract AT (04-3)-15 with the U.S. Atomic Energy Commission and by funds from the Graduate Research Committee of the University of Hawaii.

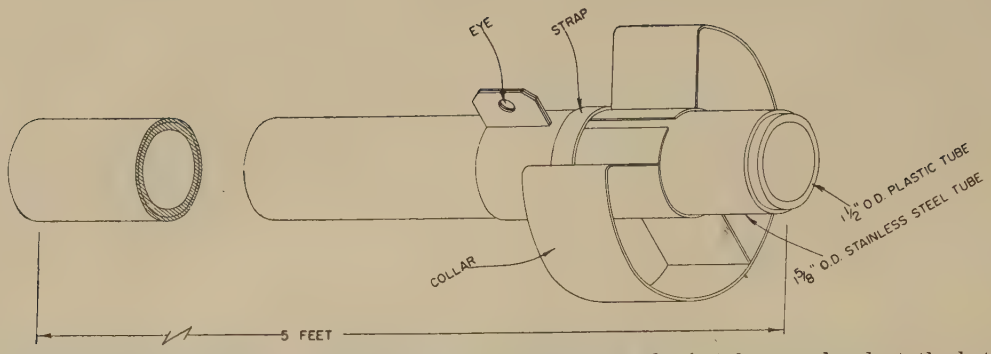

Figure 1. High-speed sampler. Both the metal outer cover and the inner plastic tube are closed at the bottom by end pieces of the same materials.

for "light" bottles. Dark bottles are made opaque by covering them with successive layers of opaque tape and black paint. This "blacking out" process should be done so thoroughly that strips of photosensitive film placed in them do not become fogged after several hours exposure to the sun.

All of our bottles are of the size and form of so-called 250 ml. acid bottles, and we have selected them to vary closely about 276 ml. in total volume. There is no reason why other sizes should not be used, and the use of smaller bottles is advantageous in some respects.

Four methods of sample collecting are in current usage:—

1. For surface samples a plastic bucket is first rinsed by dipping sea water in it. This water is discarded and the sample is then dipped. The sample water is poured from the bucket into the three sample bottles through a plastic funnel that has been rinsed with sample water just prior to filling the bottles.

2. A "snatch-bottle sampler" is used for obtaining water samples from beneath the sea surface down to depths of about 20 metres. This sampler consists of a one litre plastic bottle attached to a line and a rubber stopper attached to another line. A weight is attached to the bottom of the bottle, and, with the stopper in, the bottle is lowered to the desired depth. The stopper is then pulled out by pulling on the line attached to it and the bottle is allowed to fill. The filled bottle is then pulled to the surface. The sample bottles are filled directly from the plastic bottle.

It appears that this sampling technique gives results comparable (Table 1) to bucket sampling. The regression of about one, obtained by plotting the results from a series of samples obtained by this method at 20 metres and from the surface by a bucket (Doty & Oguri, 1957, Fig. 1), lends strength to this idea.

3. The Van Dorn sampler (Scripps Institute of Oceanography, 1955) is used to obtain samples below 20 metres and on casts where samples from several levels are desired. It consists of a plastic cylinder four inches in diameter, the ends of which, upon operation of the closing mechanism, are stoppered by two rubber plungers of the type commonly known as "plumber's helpers". This sampler has advantages over others such as operating at any depth, yielding results comparable to bucket samples (Table 1), and being of such a construction that sampling can be done with several samplers at different depths on one line.

4. A high-speed sampler (Fig. 1) has enabled sampling from commercial vessels traveling as fast as 23 knots in number 4 seas and where the deck was at least 35 feet above the water. The sampler, as indicated in the figure, is completely lined with plastic, which protrudes about one-eighth of an inch at the open end. The sampler is handled on $^{3}/_{32}$-inch stainless-steel stranded hydrographic cable. The device can be handled by hand, and, though the inboard end of the cable is always made fast, no very strong strain is placed on the equipment.

The collar shown in the figure is not used unless the vessel is of such design that the sampler tends to gouge paint from the ship's hull. Normally the water moves away from the hull of a vessel while it is under way, and this movement is usually sufficient to keep the sampler away from the hull while it is filling (a matter of seconds), but while the sampler is being brought to the deck it often swings wildly and sometimes strikes the vessel. If, when the bottles are filled, specks in them indicate the probability that material knocked off the hull is being included, the bottles are emptied and another sampler of water is drawn from the sea.

3. Addition of Radioactive Sodium Carbonate Solution

Only clean glassware should contact the stock solution of radioactive sodium carbonate to prevent contamination which may fix carbon or which, in turn, may cause loss of carbon-fourteen as a precipitate or as carbon dioxide, or which may affect the ability of the organisms to fix carbon in the sample. The use of bulk stock solutions gives less consistent results

C^{14} STOCK 4 CRUISE OR EXPERIMENT: Hugh H Smith #37

CONDITION, DATE, POSITION OR STATION	BOTTLE NO	TIME IN	TIME OUT	PLANCHET NO.	RAW COUNTS	STANDARD COUNT	RAW COUNTS STANDZD	C/M	C/M MINUS BCKGND	AVE C/M	TIME INCUB	L-D	MG C/HR/M³ OR C/M/HR
A	B	C	D	E	F	G	H	I	J	K	L	M	N
Sta 4 0800 11/21/56 178°12'W 18°07.5'N	L 137	0806	1406	9/0	1075/5	123488/5	1291	258	233	233	6 0	.89	0.1816
	L 126			9/1	1079/3		1070	387	332	332		288	0.2158
	D 51			9/2	917/6	125624/5	415	69	44	44			
Sta 5 0803 11/22/56 178°03.6'W 17°54.9'N	L 152	0810	1410	9/3	1123/6	248515	1129	188	163	15*	60	121	0.0907
	L 143			9/4	1083/6		1081	180	155				
	D 37			9/5	936/7	123509/5	439	63	38	38			

Figure 2. Data sheet used both in recording data and calculating.

than the use of steam-sterilized aliquot stock solutions in ampoules[1]). Pipetting aliquots in the laboratory can be done so that the coefficient of variation between ampoules is less than 1·5.

The top of the ampoule is broken off to initiate addition of the isotopic carbon solution. A clean glass pipette attached to an automatic delivery pipette, calibrated to deliver between 1 and 2 ml., is used to withdraw the contents of the ampoule. The solution is then released at the bottom of the sample bottle. A pipetteful of sample water is drawn from the upper half of the bottle and used to rinse the empty ampoule. The rinse water is then returned to the middle of the bottle and the pipette is rinsed with water from the top of the bottle. The bottle is then stoppered.

The amount of activity added to each sample bottle in the experiment or on the cruise is recorded on sheets specially designed to receive this and other data required by the method (see Fig. 2).

Prior to incubation the top of the opaque coated "dark" bottles must be made light-tight. This is done by folding a square piece of aluminium foil down over the stopper tightly against the neck of the bottle. If the foil is to be re-used, it must be checked each time for holes.

The inoculated bottles should be immediately placed in the incubator. The "time in" is recorded in column "C" opposite the related bottle number in column "B" on one of the data sheets (Fig. 2).

[1]) Directions for preparation both of the radioactive solutions and of the aliquots of activity in sealed ampoules are omitted here as a space-saving measure.

4. Incubation in the Light Apparatus

To obtain results the major variable of which is due to variations in the sample, it is felt the samples should be incubated under uniform conditions of

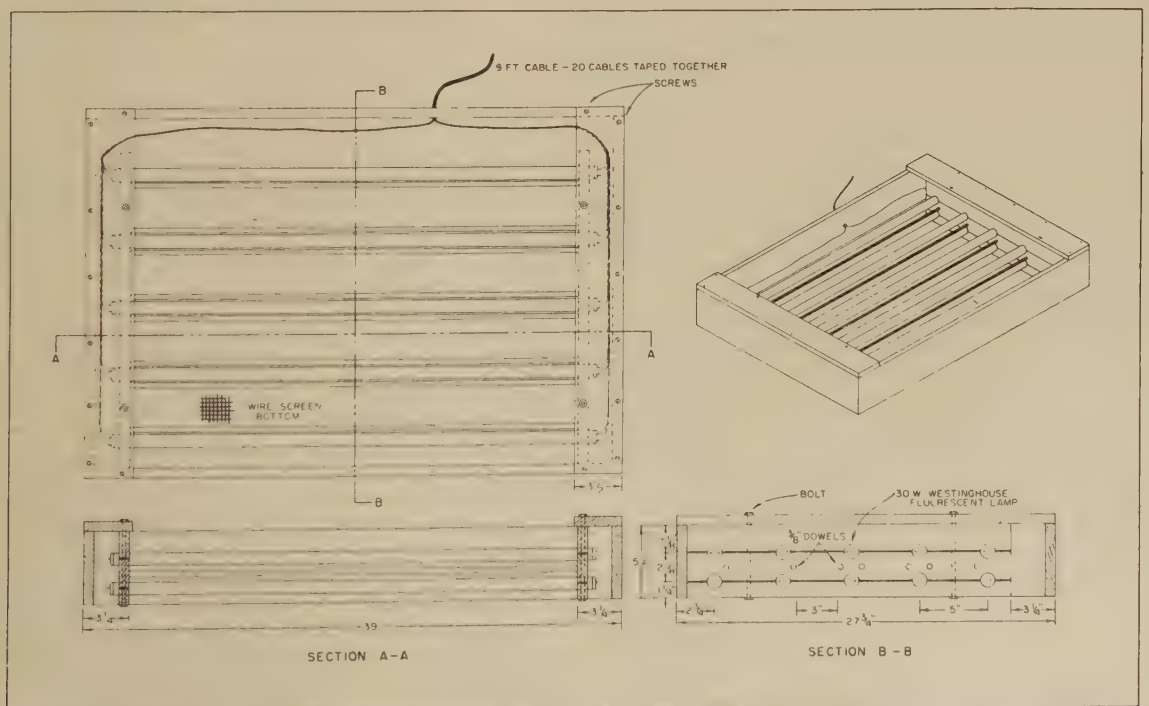

Figure 3. Incubator holding 44 bottles. This is locally referred to as the "Salpa tank" for it was first used in the live-well of this vessel. Cooling is obtained by immersing this type of tank in a live-well or water bath although, with the wire screen replaced by plywood, it may be used as a self-contained unit.

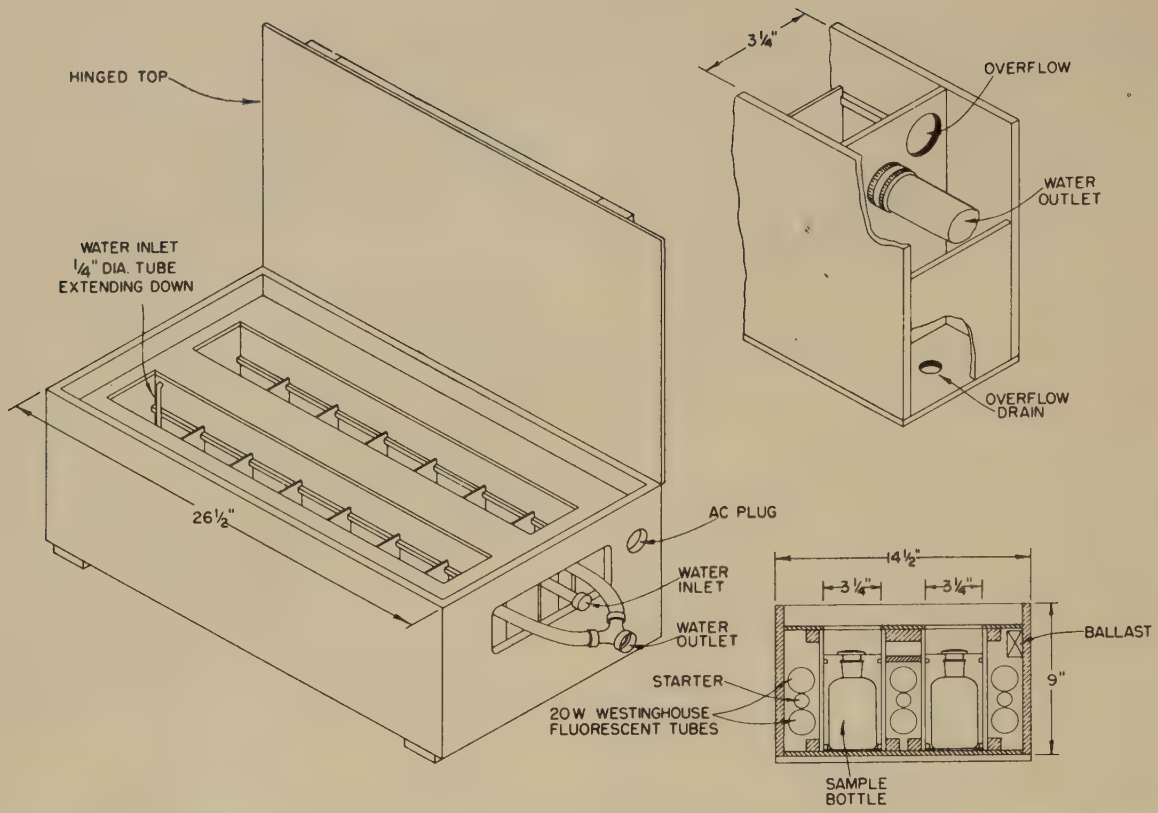

Figure 4. Incubator holding 14 bottles. This is the tank usually used at sea. The separators between the individual bottle compartments do not quite reach the side walls of the two water-containing tanks in this incubator.

light near sea surface temperature. The incubators in use (Figs. 3 and 4, and in King, Austin, & Doty, 1957, Fig. 5) are supplied with circulating water that holds the temperature to within less than one degree of that of the water supply. This temperature is about that of the water through which the ship is cruising. The light is supplied by fluorescent tubes.

Care should be exercised in selecting fluorescent tubes having the same fluorescent coating material; for the spectral emission differs with different coating substances. All the above incubators at present use Westinghouse "cool white" (circline, standard or thinline) fluorescent tubes providing an intensity of 1500 to 2000 foot candles. In general, fluorescent tubes are rather uniformly bright except for a few inches near their ends. Dark bottles are thus placed near the ends. If more samples have been collected than will fit into the incubator, dark bottles can be placed in a bucket or sink.

The period of incubation, with 5×10^6 counts per minute of carbon-fourteen in each 276 ml. bottle, should be from two to eight hours. During this period carbon fixation in the sample is approximately linear if incubation is begun sufficiently early in the morning so as to avoid periodicity effects (Doty & Oguri, 1957). A longer period is more desirable than a shorter period, since a larger amount of carbon-fourteen incorporated in the cells, as fixed carbon, will permit a shorter period for counting to appropriate statistical accuracy. Our aliquot activities and incubation period were originally selected having in mind that hydrographic stations are often six hours apart.

The question is sometimes raised as to the effect of agitation or stirring during sample collection or incubation. Steemann Nielsen (1952, p. 130) constantly mixed the water in his incubating sample bottles. Others, including ourselves, have not done so.

We thus devised some experiments to test the hypothesis that there was a relationship between productivity measured and agitation of the incubating samples. In these experiments (e.g., Table 3), with controls, samples were agitated before or during incubation and a considerable increase in fixation was usually found. Without going into the different experiments it can be stated that rather independently of the degree or the method of agitation, an increase of about 30 to 50 % in productivity was measured in each case.

We do not know whether the higher (with agitation) or lower (without agitation) figures are the more similar to what happens in nature below the sea

surface. Since the increase is relatively independent of the degree of agitation, it is suspected that the agitation merely destroys a limiting diffusion gradient near the plankton cells or affects the cell so as to enhance the rates of transport and material exchange with the surrounding medium.

Table 3
Comparison of different means of agitation with non-agitation

(L = light bottles; D = dark bottles)

Treatment	Serial treatments of the water or other conditions		% of control	Mean value for experiment
Experiment A				
Water shaken uniformly for 50 oscillations in a half-filled 5 gallon carboy before drawing each sample of the series. Control drawn before shaking began.	1	L	135	L 158
		D	105	D 104
	2	L	158	
		D	116	
	3	L	171	
		D	96	
	4	L	168	
		D	100	
Experiment B				
Water stirred with tygon-covered stirrer at about 1000 r. p. m. for successive 3-min. periods between the serially numbered samplings. Control drawn before stirring began.	1	L	96[1])	L 138
	2	L	90[1])	
	3	L	140	
	4	L	127	
	5	L	123	
	6	L	144	
	7	L	146	
	8	L	148	
Experiment C				
Water in bottl. rocked about 30 cycles per min. through an arc of approx. 60 deg. during incubation. No rocking during incubation of control.	Rocked	L	118	L 118

[1]) Not used in computing mean per cent. values.

5. Filtration

Following incubation the bottles are removed and filtered through millipore "AA" filter discs, 15/16 inch (24 mm.) in diameter, mounted in a manifold arrangement (Fig. 5) that holds three bottles, the usual complement of two light bottles and one dark bottle. There are a number of other filter holders, such as Tracerlab E-8 B, which are quite satisfactory but require more attention from the operator. Filtration of an entire bottle of sample water, under suction from an aspirator, usually requires only three to five minutes.

The time the bottles are removed from the incubator is recorded on the data sheet (Fig. 2) in column "D", as "Time out". The total time of incubation is obtained as hours and hundredths and recorded in column "L" of the data sheet.

After filtration the disc is "washed" by drawing through it about 20 ml. of 0·001 N HCl in 35 $^0/_{00}$ NaCl followed by about 30 ml. of filtered sea water. Sea water must not be used for making the acid solution since it will buffer the acid.

The filter holder is disassembled when all the liquid has passed through and each filter disc is placed in one of a number of numbered holes cut in a piece of corrugated paper board. This corrugated board is then placed in a desiccator for drying and storage. The number of the hole is recorded in column "E" as the "planchet number".

6. Washing Equipment

After each use the sample bottles are first rinsed with concentrated hydrochloric acid. They are then rinsed twice with running sea water or tap water. The first rinse should completely fill the bottle to remove acid fumes in the bottle. This is followed by two rinses with about 75 ml. of distilled water. The bottles are then stored upside down to drain, although they do not need to be dry for re-use.

The filter holders are generally washed once a day by immersing them in dilute hydrochloric acid solution (about 1 % HCl). This is quickly followed by a rinse in fresh water.

The glass pipettes are generally washed once a day; first by flushing them with concentrated hydrochloric acid and then by rinsing with distilled water several times. The samplers are similarly washed but usually the acid is the concentrated acid from the bottle washing process.

7. Planchet Storage

Our data leave open the possibility that the planchets washed with 0·01 N acid decay more rapidly than those washed with 0·0001 N acid, particularly when stored over $CaCl_2$. When silica gel is used as the desiccant there is little loss of carbon-fourteen from the planchets with time. Soda lime mixed with the desiccant removes the atmospheric CO_2 in the desiccator and reduces exchange with the carbonate in the sample.

Metal desiccators are used because they have the advantages of lighter weight and greater durability. These factors are important where planchets are being shipped by air or used at sea on research vessels which are seldom noted for their stability. Upon occasion we have airmailed dry planchets wrapped in aluminium foil, but protected from chafing, from Honolulu to New York and had them returned with no significant loss of count.

8. Counting

A filter disc (i.e., a planchet) to be "counted" is removed from the desiccator and mounted on a Tracerlab E 19 ring-shaped planchet holder. The

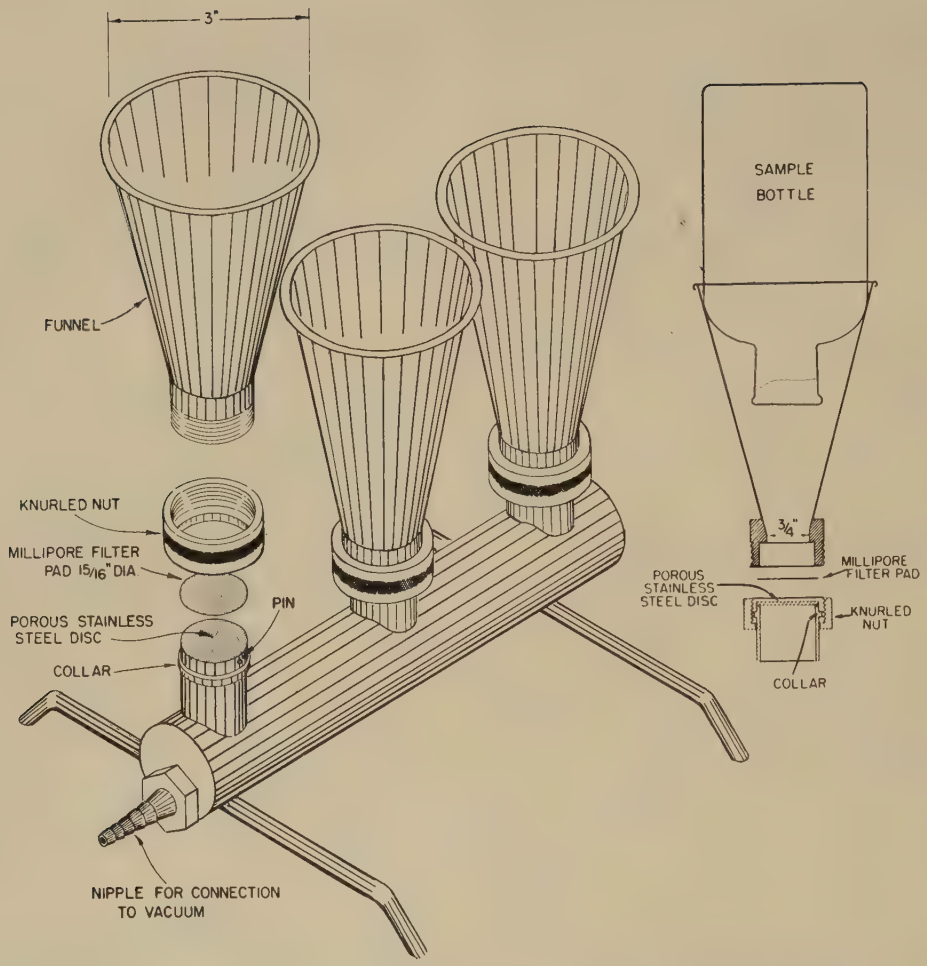

Figure 5. Filtering manifold. The inside diameter of that part of the funnels regulating the area of the filter discs used should be uniform throughout.

filter discs, i.e., planchets, are routinely mounted on planchet holders which have been lightly greased to hold them down. Manual counting is done in our laboratory with a Tracerlab SC 16 windowless gas-flow counter coupled to either a Tracerlab 1000-Scaler or a Nuclear-Chicago 161 A-Scaler. Automatic counting is done using a Nuclear-Chicago automatic sample changer with a gas-flow counter, with or without a "micromil" window, coupled to a printing recorder through the above scalers. Automatic counting has the advantage of saving a large amount of labour, the manual counting requiring the full attention of the operator. However, with an electric calculator the operator can make the calculations to complete Fig. 2 while doing the manual counting.

Instead of prepared "Q" gas, a mixture of U.S.P. XII helium bubbled through ethanol at — 5° to — 15° C. is used. The low temperature is necessary to obtain a proper mixture of helium and ethanol, and it is achieved by cooling the container of ethanol with an ice and salt mixture.

The light samples are counted, in manual counting, for 3 to 10 minutes to a minimum of 1080 total counts for a reliable error of 5 %. Dark samples are counted for 3 to 10 minutes also, although a minimum total count of 400 for a standard error of 5 % is considered acceptable. Due to pressure of time few samples are counted beyond 10 minutes, even though the desirable minimum number of counts may not have been obtained. This data, called "raw counts", is recorded in column "F" of the data sheet (Fig. 2) opposite the proper planchet number in column "E".

Every third planchet counted is a plastic disc containing carbon-fourteen. This standard is counted for five minutes. The count is recorded in column "G" in the manner illustrated. Each sample is corrected by proportion to a basic standard counting rate of 25,000 counts per minute using the standard

count immediately preceding or following it (column "H"). The proportion used, e.g., to correct the first number in column "F", is $\frac{1,275\ (125,000)}{123,480}$. The use of this proportion corrects for the variation in behaviour of the counting equipment.

If the dark sample differs in time of incubation from the light sample to any great extent, e.g., if over 20 minutes different, both counts are corrected to a standard time, usually six hours. Otherwise, the time of incubation for the light samples is accepted as the time for both light and dark samples.

The total count (column "H") is divided by the number of minutes counted (from column "F") to give counts per minute (column "I"). From this figure, background counts per minute is subtracted and the result recorded in column "J" (Fig. 2). Background counts per minute is determined daily by placing a clean filter in the counter and counting for a period of 0·5 to 1 hour and dividing the count by the number of minutes. Making background counts of each counting chamber and the different planchet holders provides a check on "spills" and the errors that would be introduced by their remaining undetected.

In cases where more than one light or dark bottle was obtained from the same sample, and these replicates were incubated for the same length of time, the values obtained for each (in counts per minute minus background) are averaged (column "K") if the difference between the lowest and highest figure obtained is not over 25 % of the highest value. When the difference is larger, the results are carried separately in column "K". Note in Figure 2, columns K, M, & N, how the data from stations 4 and 5 are treated differently in this regard.

Almost any technique accident tends to reduce the light bottle count and thus, probably, the higher count is usually the more nearly correct when replicates differ. The opposite is true in the case of dark bottles, for high counts are most often due to light leaks. Since the dark bottles have numbers on them, when an inordinately high dark count is obtained this last possibility can be checked readily by inspection of the bottle or checking other cases where the same bottle was used.

9. Calculation of mg.C/hr./m.^{3}

In the light bottle there occurs fixation of carbon by photosynthesis, exchange and other reactions (such as Wood-Werkmann reactions). In the dark bottle, since light is excluded, photosynthesis does not occur. A. H. Brown (1953) and Ryther (1954) indicate respiration to be essentially the same in darkness and light, and Ryther (1956c) has shown carbon-fourteen uptake to be essentially equivalent to net photosynthesis. The assumption is, therefore, that average light bottle counts per minute minus average dark counts per minute (column "M", Fig. 2) yields net "counts per minute" (= cpm) due to photosynthesis, i.e., "(1)",

(1) Light bottle cpm — Dark bottle cpm = Net cpm.

The addition of a known quantity (total cpm) of the tracer carbon-fourteen and the recovery of a known quantity of it (net cpm) gives a fraction (2) representing the amount of tracer carbon fixed relative to the total amount put into the sample bottles initially.

$$(2)\quad \frac{\text{Net cpm}}{\text{Total cpm}} = \text{Fraction of tracer carbon fixed.}$$

The tracer carbon fixed was fixed over a period of time, the period of incubation. If the above fraction is divided by the hours of incubation (Fig. 2, column "L"), we get the relative amount of tracer carbon fixed per hour. This fraction (3) is assumed to be proportional to the total carbon fixation in the bottle.

$$(3)\quad \frac{\text{Fraction of tracer carbon fixed}}{\text{Hours of incubation}} = \text{Fraction of tracer carbon fixed per hour.}$$

From Sverdrup *et al.* (1942) we derived a value of 90 mg. CO_2 per litre for the waters in which we work. Since the solution bearing the tracer carbon has been standardized to contain this concentration of CO_2, the assumed concentration is not altered by its addition to the sea water samples. However, the tracer substance and the ultimate result of our calculations is weight of carbon and not weight of CO_2. This is expressed as the molecular weight of carbon, 12, over the molecular weight of CO_2, 44. The fraction of tracer carbon fixed per hour is at this point assumed to be also the fraction of total carbon fixed per hour, since the addition of tracer did not alter total concentration. Therefore, to compute the total amount of carbon fixed in one litre of sample, the total CO_2 per litre is multiplied by 12/44. The amount of carbon fixed per hour per litre is then:—

$$(4)\quad \text{Fraction of tracer carbon fixed per hour} \times \frac{90\text{ mg. } CO_2}{1} \times \frac{12}{14} = \text{mg. carbon fixed/hr./litre.}$$

The calculations so far have yielded a result for one litre. To facilitate determination of productivity per square metre, the result is multiplied by 1000 to yield carbon fixed per cubic metre. A combination of all terms is then:—

$$(5)\quad \frac{\text{Net cpm}}{\text{Added cpm}} \times \frac{1}{\text{hrs of incubation}} \times \frac{90}{1} \times \frac{12}{44} \times \frac{1000}{1} = \text{mg. C/hr./m}^3.$$

When this is simplified we obtain the following equation, (6), the terms for which are obtainable from the data sheets.

$$(6)\quad \frac{\text{L—D}}{\text{Hours of incubation}} \times \text{K} = \text{mg. C/hr./m}^3.$$

This calculation we accept as yielding a raw data figure for net primary productivity (column "N", Fig. 2). No correction is made for isotope effects or growth, nor is any attempt made to compute more accurately the actual concentration of carbon dioxide in individual sea-water samples. Such considerations, while improving the accuracy of the method, are at present meaningless refinements, since it is felt that they represent corrections that lie well within the usual range of technique and sampling error.

10. Reliability

An analysis of the reliability of any technique may reveal the expected sum of the various inherent errors. The two major errors in the present technique seem to be in the manipulations and in the sampling. Without going into the details, coefficients of variation of up to 10 per cent. have been obtained between measurements obtained from successive samples from the same bucket by a skilled operator. Sources of errors of a few per cent. are several, e.g., note that in rounding off data to two places, a 2 % error may be introduced. Similarly, the same skilled operator's results have shown coefficients of variation from 11 to a little less than 25 per cent. in two series of samples drawn over the side of a slowly moving vessel. One operator going on a cruise after less than three hours of instruction took 146 paired samples over a period of 55 days. The coefficient of correlation between the members of the pairs was 0·992, indicating that the expected technical error will be negligible.

The ability of oceanic phytoplankton to photosynthesize under the conditions of uniform light in the incubators varies (Doty & Oguri, 1957) with the time of day. During that study the maximum values found for a day were from five to twelve times the minimum values. Yentsch & Ryther (1957), in seeking an explanation of this phenomenon in variations in chlorophyll content, found variations of about two times the minimum value. It appears at this time that the best method of correcting for this is to hold the samples, if collected after noon, in normal light and temperature circumstances until 0900 or some other suitable standard time the following day. The sample water can be held in half-filled carboys or in the transparent sample bottles themselves. At present it is felt that near the equator this yields more valid and reliable results than mathematically correcting results from samples collected and immediately incubated. In high latitudes where there is a less regular pulsing effect of light than in the tropics, it is expected the daily variations in photosynthesizing ability may be less and correction perhaps may be unnecessary.

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B. 4.

Extracellular Products of Phytoplankton and the Estimation of Primary Production

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Introduction

In the estimation of primary production in natural waters by the radio-carbon technique of Steemann Nielsen (1952) only carbon fixation in particulate matter is determined and total carbon fixation is underestimated to the extent that the algal cells liberate soluble organic products of photosynthesis into the external medium. Neither Steemann Nielsen nor commentators on the radio-carbon method (Ryther and Vaccaro, 1954; Ryther, 1956) appear to have considered the possibility that this source of error may be appreciable. This attitude is justified by several reports that in healthy laboratory cultures of Chlorophyceae extracellular products do not amount to more than 5 % of the total carbon assimilated (Krogh, Lange, and Smith, 1930; Roberg, 1930; Spoehr and Milner, 1949; Myers and Johnston, 1949). Furthermore, at moderate rates of photosynthesis there is good agreement between the radio-carbon method and the oxygen method of Gran (Ryther and Vaccaro, 1954) in which total photosynthesis is measured irrespective of any liberation of extracellular products. On the other hand, Braarud and Føyn (1930) found extracellular organic matter amounting to as much as 30 % of the total organic matter synthesized in pure cultures of a marine *Chlamydomonas*; recent work with Myxophyceae (Fogg, 1952) and Chlorophyceae (Allen, 1956) has shown also that, under some circumstances at least, the extracellular products in healthy cultures may amount to 50 % of the total material assimilated; Tolbert and Zill (1956) have found that glycolic acid, an early product of photosynthesis, is liberated to the extent of up to 10 % of the total carbon fixed from photosynthesizing *Chlorella* cells. In this connexion it must be remembered that if there is equilibrium between the extracellular and intracellular concentrations of a given metabolite then for a given volume of algal suspension the relative amount of metabolite present extracellularly will be greater the more dilute the suspension. In this might lie at least part of the explanation for the lower rates of photosynthesis indicated by the radio-carbon method as compared with the oxygen method in oligotrophic waters.

Clearly, it is important that direct determinations of the extent to which the extracellular products of photosynthesis are liberated by phytoplankton should be made and some work to this end is described in this paper.

Methods

Measurements of primary production have been made *in situ* at various depths in two Swedish lakes, Erken (59°50′ N., 18°34′ E.), which is eutrophic, and Torneträsk (68°25′ N., 18°50′ E.), which is oligotrophic. The method used was essentially that of Steemann Nielsen (1952), in which photosynthetic carbon fixation in samples exposed to the light in the presence of ^{14}C-bicarbonate is estimated from the radioactivity of the particulate matter retained on filtration through a membrane of 1·0 to 0·5 μ pore diameter. For the Erken experiments the technique was as used by Rodhe (in the press), the main difference from that of Steemann Nielsen being that treatment of the filter with hydrochloric acid is omitted, simple washing with water having been found satisfactory in removing inorganic ^{14}C, from freshwater phytoplankton at least. For the Torneträsk experiment the procedure was similar except that the tracer solution was not contained in sealed ampoules but was put through a membrane filter just before use, its activity being determined after this operation. Water samples and temperatures were obtained by means of a Ruttner water sampler.

Measured amounts of the filtrates from these determinations were acidified with 2 ml. N/10 hydrochloric acid per 100 ml., aerated vigorously to remove carbon dioxide, then neutralized with sodium hydroxide and evaporated to dryness on the water-bath. The solid residue from each sample was extracted four times at room temperature with small (ca. 0·5 ml.) portions of 80 % aqueous ethanol and the combined extracts were dried as evenly as possible on a copper or stainless steel disc. In this way all water-soluble non-volatile organic material in each filtrate was obtained in a form suitable for assay of its radioactivity. The residue left after extraction showed no appreciable radioactivity. The filtrates from the Erken experiments were dealt with immediately but those from Torneträsk were preserved by addition of 0·75 ml. of 3 % mercuric chloride in N hydrochloric acid and assayed on return to England.

The radioactivities of the materials on the filters and discs were determined using a gas-flow counter. The counts reported are corrected for background activity and for self-absorption, which for the samples obtained from the filtrates was from 58 to 66 %. Slight differences in geometry which occurred were found to have no appreciable effect on counting rates. The standard deviations given are those for counting error only.

Total inorganic carbon concentrations in the lake waters were determined from pH values measured electrometrically and measurements of titration alkalinity, using dissociation constants given by Harvey (1955).

Results

Rate of photosynthesis per unit volume of water was found in these experiments to vary with depth in the usual way. The results for Lake Erken given in Table 1 indicate, when a correction of + 10 % to allow for isotope discrimination and loss of ^{14}C by respiration has been made (Steemann Nielsen, 1952), a total fixation in particulate matter of 0·57 g. carbon per m². per 24 hrs. At this time *Gloeotrichia echinulata* was the predominant phytoplanktont. Appreciable radioactivity was found in the filtrates and was considerably higher for the samples which had been in the light than for the dark control. For most of the photic zone the extracellular fixation was less than 2 % of that in the particulate matter. In the whole water column the extracellular products of photosynthesis amounted to 1·55 % of the intracellular products and the total production was therefore 0·58 g. carbon per m². per 24 hours. The relative fixation in extracellular products did not remain constant with depth; the value of 0·83 % at 2 metres is significantly lower statistically than those for samples above and there was a fairly regular increase at depths below 2 metres to a value of 8·3 % at 10 metres. Fixation in dissolved material in the dark was relatively high.

Similar results to these were obtained in a second experiment carried out on 2.—3.IX.1956 when *Aphanizomenon flos-aquae* was replacing *Gloeotrichia* as the most abundant species in Erken. Fixation in particulate matter was again about 0·57 g. carbon per m². per 24 hrs. but that in the filtrates was somewhat higher, being 2·4 % of that in particulate matter for the whole water column. This gives the total fixation as 0·585 g. carbon per m². per 24 hrs. This time there was no clear indication of a minimum relative fixation in extracellular products around 2 metres but again there was a regular increase at depths below this, the value at 10 metres being 15·7 %. However, fixation in extracellular products in the dark was this time only 2 % of that in particulate matter.

The results of the Torneträsk experiment (Table 2) are less clear cut but are in general agreement with those just described. Phytoplankton was extremely sparse, chlorococcoid "μ-algae" being the chief forms with occasional cells of *Asterionella*, *Fragilaria*, and *Melo-*

Table 1
Fixation of radio-carbon supplied as $NaH^{14}CO_3$ in particulate matter and in filtrates of water samples from Lake Erken

Samples exposed from 20.00 hours 29.VIII.1956 to 19.15 hours 30.VIII.1956 at the permanent buoy 0·5 km. N. of the laboratory. Weather moderately overcast. Tracer added to give 971,000 c.p.m. per 130 ml. bottle, pH ca. 8·15; titration alkalinity 1·85 m.e./litre; total inorganic carbon content, 22·4 mg./litre.

Depth (metres)	Temp. (°C.)	Radioactivity, c.p.m./100 ml. Particulate matter (*a*)	Filtrate (*b*)	*b* as % of *a*
0·1 ...	—	5350 ± 36	104 ± 4·5	1·95
0·5 ...	15·35	7445 ± 43	150 ± 5·6	2·02
1.....	15·35	6420 ± 41	110 ± 5·1	1·72
2.....	—	3382 ± 29	28 ± 4·3	0·83
3.....	—	1794 ± 14	29 ± 3·9	1·62
4.....	15·3	938 ± 8·5	14 ± 3·5	1·49
5.....	15·3	364 ± 4·1	10 ± 3·6	2·75
10.....	15·25	120 ± 3·5	10 ± 4·2	8·33
10..... (dark)	—	95 ± 2·3	20 ± 3·9	21·0

Table 2
Fixation of radio-carbon supplied as $NaH^{14}CO_3$ in particulate matter and in filtrates of water samples from Torneträsk

Samples exposed from 17.00 hours 21.VIII.1956 to 9.20 hours 23.VIII.1956, 2·5 km. N. of Abisko Tourist Station. Weather mainly heavily overcast with rain and rough water. Tracer added to give 440,000 c.p.m. per 135 ml. bottle, pH ca. 7·4; titration alkalinity 0·235 m.e./litre; total inorganic carbon content, 3·105 mg./litre.

Depth, m.	Temp. (°C.)	Radioactivity, c.p.m./100 ml. Particulate matter (*a*)	Filtrate (*b*)	*b* as % of *a*
0·1 ...	6·7	258 ± 6·2	17·7 ± 7·8	6·8
5.....	6·5	280 ± 6·5	9·5 ± 10·9	3·4
10.....	6·4	106 ± 3·1	12·2 ± 10·9	11·5
20.....	6·0	19 ± 1·4	13·7 ± 12·8	72
30.....	5·6	11 ± 1·3	4·4 ± 7·7	40
40.....	5·6	2·5 ± 1·2	21·5 ± 12·8	859
dark[1]) .	—	1·6 ± 0·8	18·4 ± 3·5	1150

[1]) Mean of 4 samples from different depths.

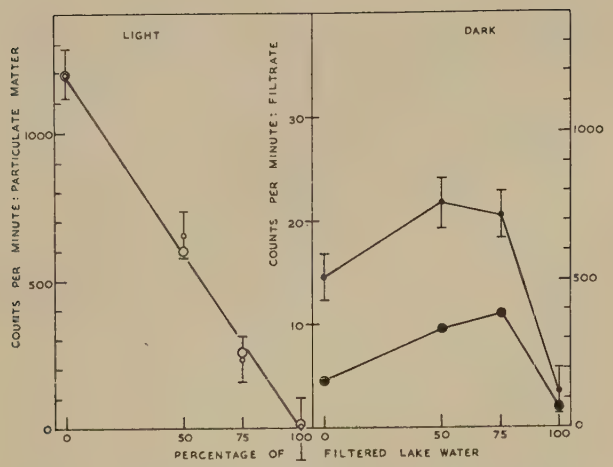

Figure 1. Fixation of radio-carbon supplied as $NaH^{14}CO_3$ in particulate matter and in filtrates from various mixtures of filtered and unfiltered water from Lake Erken. Tracer added to give 971,000 c.p.m. per 130 ml. bottle; the values given are c.p.m. per 100 ml. lake water.

○ particulate matter, light ● particulate matter, dark
∘ filtrate, light • filtrate, dark

The standard deviations of the counts of filtrate preparations are indicated by vertical lines. Note that counts for particulate matter and filtrates are plotted on different scales adjusted so that the points for 100 % unfiltered water exposed to light coincide. For other details, see the text.

sira. Because of unfavourable weather conditions the samples were left in the lake for 40 hrs. The radio-carbon method has been found to be unreliable with samples exposed for more than 24 hrs. (Ryther and Vaccaro, 1954) but at the low temperatures which prevailed in this experiment, metabolic processes involving ^{14}C-labelled products would have been slow so that the errors arising from the long exposure may not have been serious. The total fixation in the period, which was mainly overcast, was 0·032 g. carbon per m^2. The activity of the filtrates being low and self-absorption high, the individual counts for these are scarcely distinguishable statistically from the background count but the results as a whole show a relatively higher fixation in the filtrates than in the Erken experiments. However, the extracellular fixation in samples exposed to the light was not evidently greater than that in the dark and it is thus impossible to distinguish between radio-carbon incorporated in respiratory intermediates or compounds capable of undergoing exchange reactions with carbon dioxide and that incorporated in direct products of photosynthesis. At the most, photosynthetic fixation in extracellular products in this instance could have been of the order of 11 % of that in particulate matter in the whole water column. It may be noted here that offshore surface water from Torneträsk has been found to contain an appreciable amount of dissolved organic matter. An examination by the methods of Fogg and Westlake (1955) gave about 0·18 mg. total organic nitrogen and 0·021 and 0·033 mg. α-amino nitrogen before and after hydrolysis respectively, per litre.

Besides these observations *in situ*, laboratory experiments have also been made with samples of lake water. One such experiment was designed to determine how the amount of extracellular photosynthetic products was related to the concentration of algal cells. Water taken at 1 metre in Lake Erken was filtered through a membrane filter, a treatment which did not appreciably alter either its reaction or titration alkalinity, then mixed in varying proportions with unfiltered but otherwise similar water containing phytoplankton in which *Gloeotrichia* and *Aphanizomenon* were the predominant forms. Photosynthesis in these mixtures was determined by the usual radio-carbon technique over a period of 6 hrs. during which the bottles were incubated at 19·6° C. in a light intensity of 700 lux from fluorescent tubes. It is clear from the results, which are given in Figure 1, that photosynthesis per unit volume of water was proportional to phytoplankton concentration. Fixation of radio-carbon in the filtrate was about 3 % of that in the particulate matter, irrespective of plankton concentration. This provides confirmation of the supposition that the observed radioactivity in the filtrate preparations from Erken water exposed to the light is due to radio-carbon incorporated in extracellular products of photosynthesis and not to incomplete removal of inorganic carbon or exchange reactions of dissolved organic substances having no direct connexion with photosynthesis. Fixation in the filtrate from bottles kept under otherwise similar conditions in the dark was relatively higher than that in the light, ranging from 5·3 to 8·3 % of that in the particulate matter. The peculiar variation of dark fixation, with the highest rates at intermediate plankton concentrations, suggests interactions between phytoplankton and bacteria which deserve further investigation.

Discussion

The results obtained in this investigation show that, in experiments lasting for 6 hrs. or more, appreciable proportions of the carbon fixed in photosynthesis by natural phytoplankton populations may be liberated from the cells in soluble form and thus that methods in which fixation in particulate matter only is determined underestimate to some extent the total fixation of carbon in natural waters. It is to be expected that the method which has been used in this investigation for obtaining the organic matter dissolved in lake water in a form suitable for assay of its radioactivity would give quantitative recovery of such extracellular products of algae as have so far been chemically identified, i.e., polypeptides, pentoses (Fogg, 1952), glycolic acid (Allen, 1956; Tolbert, 1956), oxalic and pyruvic acids (Allen, 1956). Volatile products and substances such as proteins which might yield

insoluble derivatives under the treatment used would not be recovered but there is nothing to suggest that such substances are liberated in appreciable quantities by algae and it appears that the method gives a reasonable estimate of total fixation in extracellular products. It would be desirable to check this conclusion using a wet combustion method as a means of recovering the carbon present in dissolved organic form in lake water. The relatively low values that have been obtained for fixation in extracellular products in lake waters exposed to light therefore indicate that the error introduced into productivity determinations by considering fixation in particulate matter only is not a major one. This conclusion is in quantitative agreement with the results of the earlier work on the liberation of extracellular products by algae to which reference was made in the introduction. However, there are indications that the relative amount of extracellular products may vary according to circumstances and the possibility that it may sometimes be substantial must be discussed.

The possibility that the relative production of extracellular substances may be inversely related to cell concentration does not appear to be realized, at least, with blue-green phytoplankton. This is in agreement with results obtained by Fogg (1952) for the extracellular polypeptides of *Anabaena cylindrica* in pure culture.

There are indications that the relative amount of extracellular products of photosynthesis liberated is dependent on light intensity. In Erken, below 1 metre, photosynthesis was proportional to light intensity (as shown by the approximation of the points to a straight line when log. carbon fixed is plotted against depth). In this range there were decreases in the relative fixation of carbon in the filtrates with increasing light intensity, the highest values being found, with one exception, in the dark bottles. This is in agreement with the results of Käńdler (1954), who found uptake of sugars from the external medium by *Chlorella* to be increased in the light, and of Fogg (1956), who found the proportion of carbon fixed in the water-soluble fraction of *Navicula* cells to decrease as light was increased up to intensities approaching saturation. Allen (1956) concluded that accumulation of soluble organic products in cultures of *Chlamydomonas* spp. was favoured by high light intensities but if comparison is made between cultures at equivalent stages of growth, which according to Fogg (1952) is the only valid way in which comparison can be made between cultures grown under different conditions, then, in fact, her results show greater amounts of extracellular organic matter in cultures grown at low light intensity. Fogg (1952) found that variation in light intensity was without effect on the relative amount of nitrogenous substances excreted by *Anabaena* but these substances are not likely to have any close relationship to intermediates in photosynthesis and their production would not be expected to be directly affected by light. An increase in production of extracellular substances occurring at low light intensities might be of appreciable ecological importance when a substantial phytoplankton population is carried downwards by turbulence or is subjected to overcast weather. The apparently relatively higher fixation in the Torneträsk filtrates as compared with those from Erken might be ascribed to the fact that the former were taken up after overcast conditions had persisted for almost 24 hours.

The effect of light of greater than saturating intensity is not clear, although in the results obtained there is an indication that it produces an increase in relative fixation in the filtrates. If this really does take place it would be consistent with the finding that in *Navicula* carbon fixation in the water-soluble fraction of the cells increases when light intensity is increased above saturation (Fogg, 1956). However, it does not seem that the increased escape of photosynthetic products from the cells of phytoplankton at high light intensities is sufficient to account for the generally observed inhibition of photosynthesis in the surface waters of lakes and the sea.

Of the effects of other factors little can be said at present. Considerable amounts of extracellular substances are sometimes produced in algal cultures and until further information about the factors responsible for this is obtained it cannot be excluded that corresponding amounts of material are liberated under some circumstances in nature. It is also likely that there are important differences between species in the extent to which such substances are liberated.

Even though the liberation of extracellular products may be slight when considered as a possible source of error in the estimation of primary production, the process may nevertheless be of some biological importance. The indication that the phytoplankton of Torneträsk produces appreciable amounts of extracellular products and that a quite high concentration of dissolved organic matter is present in the water lends support to the suggestion of Rodhe (1955) that the "μ-algae" of sub-arctic lakes are able to exist chemotrophically during the winter darkness using dissolved organic matter produced during the previous light period. It must also be remembered that methods such as those used in this investigation do not necessarily give an estimate of the actual amount of material lost from the phytoplankton cells in dissolved form. Dissolved substances of low molecular weight would probably be avidly absorbed by other plankton so that the existence of an equilibrium between intra- and extracellular concentrations of such substances in phytoplankton might not be apparent under natural conditions. The theory of Pütter (1908) that extracellular products of algae form a major source of food for some marine animals might well bear re-examination by modern techniques.

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B. 5.

Investigations of the Chemical Basis of Productivity in Sea Basins

By M. V. Fedosov

The "oxygen method" of determination of photosynthesis and the formation of new organic substance which is preferred by physiologists is also applied in the study of the productivity of sea basins. This method is based on the biochemical reaction of photosynthesis of a plant cell — production of oxygen in the synthesis of organic substance from carbon dioxide and water.

Two basic variants of carrying out the oxygen method of determining the intensity of photosynthesis and the production of organic substance in the investigations of natural waters are used: the "bottle" method and the method of direct observations in the sea (*in situ*). The latter has been applied on a larger scale in the investigations of the sea.

The first thing the method allows us to do is to determine the spatial and qualitative differences both in the photosynthetic effect and in the processes of oxygen consumption. The method of direct observation in the sea is more acceptable in naval expeditions, as it gives mass material and good mean values for a given area and depth zone of the sea.

Theoretical calculations and experimental data allow us to come to the conclusion that the application of the coefficient $0{\cdot}87 \pm 0{\cdot}07$ for the recalculation of the results of determinations of the daily fluctuations in the oxygen content of sea water leads to values corresponding to the real growth of organic substance of phytoplankton with the indicated degree of accuracy.

Daily stationary observation in the sea from a ship is the most essential method of getting the most complete material on the dynamics of the content of oxygen in sea water in a given area.

Two contrary biochemical processes take place in sea water: 1. production of oxygen in water as the result of photosynthesis; 2. consumption of oxygen for the oxidation of organic substances during their decay. The observations are conducted on oxygen production in water, i.e., increase of its content in sea water in the daytime and consumption of oxygen at night.

$$-O_2 = \bar{\Delta}\,O_2 \text{ during the night} \times \frac{24}{24 - \text{hours of the day}}$$

Consumption of O_2 during twenty-four hours — Loss of O_2 in water during the night (from sunset till sunrise) — Duration of dark (night) time (from sunset till sunrise)

$$\Sigma + O_2 = \bar{\Delta}\,O_2 \text{ during the night} \times \frac{24}{24 - \text{hours of the day}} + \dot{\Delta}\,O_2 \text{ during the day}$$

Production of O_2 — Growth during twenty-four hours

It is convenient to apply the following regime of observations for carrying out these observations in expeditionary conditions at sea when there is no possibility to stay for twenty-four hours at one place.

All the material on the content of oxygen in sea water for a given region of the sea are divided into the groups:—

1. The content of oxygen in sea water is observed just before sunrise. Taking into consideration the hysteresis of photosynthesis, the results of the observations for the whole period of the sunrise (before and after) are practically the same.

2. The content of oxygen is observed in the period of the post-afternoon maximum of the daily development of the photosynthesis. The hours of this period as well as those of the other periods are different for the seasons and geographical areas of observations.

3. The content of oxygen is observed just after sunset. The phenomenon of hysteresis of the foregoing photosynthesis compels us to take the mean value of the content of oxygen in water during one or two hours after sunset from several observations.

The time (hours) of the first and third term is easily determined according to a reference book. It is necessary only to check the influence of hysteresis of

the photosynthetical process. In the former case and during the first one or two hours after sunrise, the observations give a daily minimum of the oxygen content as exact as if it was immediately before sunrise.

In the latter case (the third term of observations) of the hysteresis of the photosynthesis also the mean value is taken of several observations taking place during the sunrise and one or two hours after it.

The time (hours) of the second group of observations in the post-afternoon period (the maximum value of the sun radiation) is determined experimentally for each sea region and season according to the data of half-daily continuous observations from sunrise till sunset. In many cases this period goes on from 14 to 21 o'clock with a maximum of the oxygen content during 15—18 o'clock.

The mass material of observations on the oxygen content in sea water gives sufficiently reliable mean values of the daily minimum and maximum for the productive layer of the given sea region.

However, in all cases it is necessary to control by daily continuous observations the hours of the periods mentioned.

The material of mass observations in the period directly after sunset and twilight give a control figure for the determination of the intensity of respiration by night, when the contrary process of photosynthesis is absent or goes on very slowly.

The results of these observations should be controlled by the so-called "bottle method" of determination. This method can easily be carried out on an expeditionary ship if a sterilizer for bottles and water thermostats for various temperature levels (corresponding to the water-layer temperatures in the sea) are available for the incubation of the bottles with the samples of the water investigated. The application of the "bottle method" becomes complicated only for the bottom layer of shallow sea water, as the consumption of oxygen for the oxidation of the organic substance of the sea bottom is limited in bottles whereas in reality sometimes goes on very intensively.

Using the results obtained from the observations referred to we can compute and determine:—

1. The intensity of the formation of new organic substance of phytoplankton.
2. The value and the sign of the daily change of the balance of the organic substance in the sea, determining the correlation between synthesis and decay of the organic substance in sea water.
3. The intensity of decay of the organic substance in the sea.
4. The regions of intensive primary production in water masses with the estimation of their thickness.

The carrying out of daily stationary observations in the sea on an expeditionary ship when oceanographic work it conducted on a large scale does not take much time. Under these conditions it is more expedient as it was mentioned to use the method of mass observations in three periods during twenty-four hours, generalizing the results for each typical region of the sea.

It is convenient to determine and outline the regions of sea waters of the productive layer on the basis of collecting material according to the degree of supersaturation of oxygen in the waters of the productive zone. This work is especially easy to carry out, when the ship is in motion and with application of continuous oxigenometer.

To facilitate carrying out all the above-mentioned observations the following variant is used: mass observations are made only in the period of sunrise (directly before and after it) and in the period of the post-afternoon maximum of the oxygen content in water. The second period for the productive time in the Sea of Azov was experimentally determined by daily observations from 15 to 21 o'clock. Some shifts in time for the maximum intensity of photosynthesis will take place in each region, and in this case the corresponding hours must be experimentally determined.

In this variant of work the calculation of daily value of forming new organic substance of phytoplankton is made by the formula

$$\underset{\text{Daily production of } O_2 \text{ ml./l.}}{\Sigma + O_2} = \underset{\text{from morning minimum till post-afternoon ml./l. of maximum}}{\overset{+}{\Delta}\, O_2 \text{ max.}} \times 1{\cdot}6$$

Coefficient 1·6 is set experimentally for the central part of the Caspian Sea and the Sea of Azov from the correlation:—

$$\frac{\text{Daily production } \Sigma + O_2}{\overset{+}{\Delta}\, O_2 \text{ max.}} = 1{\cdot}6$$

It is necessary for the other seas to check this coefficient and to find its corresponding significance. Subsequent recalculation is made of the production of oxygen during twenty-four hours in ml./l. on production of organic substance in mg./l.

Such a method of work and calculation makes it easier to collect mass material and receive satisfactory data on the estimation of the intensity of the synthesis of the organic substance in the given region of the sea and for a definite term.

Hence it follows that it is very important to get mass material of observations on the content of oxygen in the upper photosynthetic zone of the sea. The results received are very valuable. They characterize the intensity of the synthesis and the decay of organic substance in the sea.

Besides, the results of observations on the supersaturation of sea water of the upper zone by oxygen, allow us to pick out regions of intensive (positive) synthesis of new organic substance as the first stage of a food chain in the sea.

Table 1

	Thousands of tons			
	River run-off	Sea	Internal turnover	% of river run-off to the value of the internal turnover
The Sea of Azov:				
Phosphorus general	13·2	—	180·0	7·4 %
Including mineral dissolved compounds	2·0	2·2	—	1·1 %
Nitrogen general	77·5	—	1,125·0	6·9 %
Including mineral dissolved compounds	6·2	32·0	—	0·5 %
The Caspian Sea: (the northern part)				
Phosphorus general	27·2	32·0	to 276·0	not less than 10 %
Nitrogen general	278·5	417·0	to 2,300·0	not less than 12 %

With the help of a comparative method one can judge of the intensity of the photosynthetical process in the given region.

The sea waters in which daily growth of a newly synthesized organic material does not take place are not usually saturated by oxygen by more than 96-99 % (mean 97·5 %). When the intensity of photosynthesis begins to increase and prevail over the decay of organic substance, the saturation of the upper layers of sea water begins also to increase reaching a certain degree of saturation.

Numerous observations referred to showed that:—

in the Caspian Sea the total supersaturation of water by oxygen reaches 107—123 %,
in the Sea of Azov: 120—150 %,
in the Aral Sea: 105—114 %,
in the Baltic Sea: 103—115 %.

At the same time biochemical consumption of oxygen in these and the other seas is expressed by the following values:

	ml. O_2/l./5 days
The northern Caspian Sea	0·76 —1·59
The central Caspian Sea	0·68 —1.55
The Baltic Sea	0·87 aver. and less
The Norwegian and the Greenland Seas	0·005—0.01

In addition to these investigations experimental work on the biochemical consumption of oxygen and the intensity of photosynthesis in various samples of water with the application of fractionation would make it possible to get material allowing us to determine separately the components of oxygen-consuming sea water, to assess the physiological state and the characteristics of living organisms, to give the characteristic of the "turnover" of nutrients and of organic substances in the sea with the quantitative estimation of all correlations.

The study and determination of the balance of the principal biogenic elements in sea basins is of great interest and importance in connexion with the problem of primary productivity and feeding matter.

The balance method is most important and gives good results for inland seas, sea bays, and geographically isolated regions.

When this method of the study of home seas is applied the results show, for instance, that the yearly supply of nutrients from the land in the Sea of Azov is consumed by phytoplankton eightfold or tenfold. Quite another regime of production of organic substance is observed in the waters of the Aral Sea. In this basin the phytoplankton develops hardly by more than the yearly supply of nutrients, discharged by river water.

The component of the balance of the biogenic elements in a sea basin are mainly formed of: (1) delivery of nutrients coming with water from the land, (2) delivery of matter through atmospheric precipitation, (3) exchange of matter with waters through the adjoining straits, (4) transition into the ground deposits.

Materials on separate components are still poor both in long-term and yearly aspect. The delivery into the Baltic Sea of biogenic substances of this sea basin is not studied well. Atmospheric precipitation as a source of supply of sea basins by nitrous combinations is not sufficiently studied from the quantitative point of view.

However, it is already now possible to determine the order of the elements of the balance of nutrients in a number of sea basins and bays.

In addition to external balance of nutrients of the basin the "turnover" of biogenic elements and organic substance in the sea itself is directly connected with the process of continuous formation of new organic substance and it forms its internal balance. The internal balance of the sea reflects the intensity and the result of usage of biogenic elements by synthesized water organisms which enter with the river run-off and atmospheric precipitation.

The process of photosynthesis and the process of formation of primary organic substance from mineral components are indispensable components of the internal balance of the biogenic elements in the sea.

The "turnover" of biogenic elements is composed of the following processes: (1) consumption of biogenic elements during photosynthesis, in some cases during chemosynthesis, (2) formation of suspensions by biochemical and physico-chemical method, (3) settling of these suspensions to the bottom, (4) decay and mineralization of autochthonous and allochthonous

organic substance in the sea, (5) dissolution of suspensions and components of ground deposits, and (7) their reverse transition to the water layers of the sea.

The internal "turnover" of biogenic nutrients in the sea also finds quantitative expression on the quantitative base of the external balance processes of biogenic elements.

The investigations referred to yielded the material which allows us to determine the order of correlations of the values of the external and internal "turnover" of nutrients in some of the sea basins (see Table 1).

It is clear already from this order of values how intensively photosynthetical production of plant organic substance, which is the main feeding stuff, takes place in these basins in relation to potential productivity. Both the oxygen method to the study of the productivity of the seas and the balance method are based on the materials of chemical-analytical analysis.

In respect of the determination of the content of biogenic elements of sea waters one should recognize the value of the most advanced methods of objective colorimetry without corrections with application of photoelectric colorimeters and summary determination of nitrogen compounds; these methods were available at the beginning of international oceanographic research.

The variants of the oxygen method of determination of the intensity of production of organic substance referred to in this paper are worked out on the basis of works previously carried out by PJUTTER and GRUN and the Soviet researchers: Professors BRUJEVICH, VERESHTSHAGIN, WINBERG, SKOPINTSEV, and DATZKO.

At the present time it is necessary to organize mass collection of material for more complete solution of the problem of primary productivity.

B. 6.

Quelques Questions concernant les Méthodes de l'Investigation de la Production Primaire du Plancton, basée sur des Travaux exécutés en URSS

Par G. G. Winberg

Les recherches qui avaient lieu en URSS donnent la possibilité de faire certaines considérations sur les méthodes dont on se sert pour mesurer la valeur de la production primaire du plancton.

Il s'agit de trois méthodes:—

1. Le méthode d'oxygène, bien connue, liée avec l'exposition des bouteilles assombries et claires dans les bassins.

2. Le calcul de la production primaire du plancton d'après les variations diurnes d'oxygène dissous dans l'eau. Le principe de cette méthode a été proposé par Pütter (1924).

S. V. Brouévitch (1937) a élaboré cette méthode et elle a été employée sur la Mer Caspienne, la Mer d'Azov et dans les bassins des eaux douces.

3. La méthode radio-carbonique de Steemann Nielsen dont on se sert en URSS après la modification de Sorokin (1956).

En Union Soviétique les recherches quantitatives de la production primaire du plancton ont été exécutées premièrement dans les bassins des eaux douces. Les mesures systématiques de la photosynthèse du plancton sur les lacs ont été entamés par nous en 1932 à l'aide de la méthode d'oxygène à la Station Limnologique de Kossino (aux environs de Moscou).

Ces études ont été prolongées par nous et par d'autres auteurs sur différents bassins de l'URSS (Winberg 1934; Winberg et Ivanova, 1935; Winberg, 1956, etc.). A présent la production primaire du plancton d'un grand nombre de lacs et d'étangs est déjà mesurée à l'aide de cette méthode.

Ici nous ne pouvons pas mentionner les résultats nombreux de ces travaux. Il faut noter seulement que les valeurs brutes de la production primaire du plancton des lacs sont en accord avec les résultats obtenus actuellement dans d'autres pays (Rodhe à Suède, Ohle et Gessner en Allemagne, Steemann Nielsen à Danemark). La production primaire annuelle du plancton dans les lacs, d'après nos propres données est le plus souvent 1000—2500 cal./m².an ou à peu près 100—250 g. C/m².an; dans les lacs les plus fertiles peut atteindre 400 g. C/m².an (Winberg, 1956).

La grande expérience que nous avons dans l'application de cette méthode sur les lacs et les étangs d'une fertilité différente nous oblige de reconnaître que cette méthode est bien utile pour la détermination de la production primaire du plancton dans les cas où l'intensité de photosynthèse du plancton est assez grande, par exemple — plus que 0·2 mg. O_2/l. par jour. Des nombreux auteurs pensent que la source principale des fautes est liée avec l'accroissement du nombre des bactéries dans l'eau renfermée dans les bouteilles. En réalité, beaucoup de fois il a été constaté que le placement de l'eau dans les bouteilles mène vers un grand accroissement du nombre de bactéries. Mais ces résultats ont été reçus à l'aide de numération des colonies bactériales cultivées sur le milieu alimentaire. Par cette méthode on détermine seulement le nombre des formes saprophytiques des bactéries et non pas la valeur totale.

Un résultat tout à fait différent est obtenu quand on détermine le nombre des bactéries par la méthode de numération directe. Numération directe des bactéries aquatiles a reçu une large application en Union Soviétique.

Ainsi on a appris que la quantité totale des bactéries aquatiles est 100 fois, et dans certaines eaux 1000 fois plus grande que le nombre des formes saprophytiques.

Il a été montré de même que la vitesse de consommation d'oxygène dans les eaux polluées est proportionelle au nombre total des bactéries obtenues à l'aide de numération directe et non pas au nombre des bactéries saprophytiques.

D'après nos données dans les eaux pures et les eaux polluées à 20° C. une bactérie consomme en

moyenne 0·10—0·25 $\times 10^{-9}$ mg. O_2/jour (Winberg et Lomonossova, 1935).

La détermination du nombre total des bactéries par la méthode de la numération directe a montré que dans les cas où les bouteilles sont remplies d'eau non filtrée et il n'y a pas de changement de température, le nombre des bactéries dans les bouteilles claires et foncées ne change pas durant les premiers vingt-quatre heures ou bien ce changement n'est pas considérable. C'est pourquoi il n'y a pas de raison de s'attendre à une grande consommation d'oxygène.

Ainsi, la faute qui peut être évoqué par l'accroissement du nombre de bactéries dans les bouteilles ne peut pas être de grande signification quand l'intensité de la photosynthèse est grande. Mais en ce qui concerne les eaux d'extrême oligotrophie comme les eaux tropicales océaniques cette question exige une étude spéciale.

Dans les investigations marines en URSS la méthode d'oxygène a été peu appliquée. Déjà en 1933 P. Chirchov (1938) à bord de "Tcheluskine" a fait quelques mesures d'intensité de photosynthèse sur les Mers de Kara et Orientale Sibérienne. Pendant l'exposition des bouteilles dans les vaisseaux remplis d'eau à la température proche de 0° C., la photosynthèse du plancton de la Mer de Kara a donné 0·0009—0·059 ce qui fait en moyenne 0·018 ml. O_2/l./h. (15—29 août).

Dans la Mer Sibérienne Orientale la valeur moyenne obtenue par quatre mesures est 0·053 ml. O_2/l./h. (9—11 septembre).

Sur la base de ces données et en faisant quelques admissions P. Chirchov a conclu que la production primaire brute du plancton dans la Mer de Kara fait 420 et dans la Mer Sibérienne Orientale 530 g. de "glucose" sur 1 m^2. par an. Il est bien certain que ces valeurs qui sont obtenues sur la base des analyses peu nombreuses ne peuvent pas être considérées comme bien fondées. Elles ne présentent qu'un intérêt historique comme exemple du calcul de la production primaire du plancton par la méthode d'oxygène.

M. A. Dobrjanskaya en 1950/51 a examiné la production mensuelle à l'aide de la méthode des bouteilles dans la baie de Sébastopol sur la Mer Noire (1954). Elle déclare que l'intensité de la photosynthèse du plancton dans les couches supérieures des eaux est la plus grande en avril (0·85), en mai elle atteint 1·30 et en juin 1·38 ml. O_2/l./ jour.

Mais déjà à la profondeur de 10 mètres la photosynthèse se produit avec une intensité très reduite (0·06—0·07 ml. O_2/l./ jour).

En septembre et en octobre 1954 V. G. Bogorov et R. V. Beklémichev se sont servis de la méthode d'oxygène pour la détermination de la production primaire du plancton des couches supérieures des eaux dans la partie nord-ouest du Pacifique et ils ont montré les différences des régions investiguées.

Il est bien connu, que la méthode d'oxygène possède une sensibilité trop faible pour être appliquée dans les eaux océaniques où la méthode radio-carbonique de Steemann Nielsen est préférable. Pourtant pour les eaux côtières ou pour les eaux dans lesquelles l'assimilation de CO_2 par le plancton se produit avec une grande vitesse, la méthode d'oxygène conserve complètement sa signification. Le plus grand avantage de cette méthode en comparaison avec la méthode de Steemann Nielsen consiste en ce qu'elle donne la possibilité d'évaluer non seulement de la production nette mais aussi la production brute et pour la détermination de la vitesse de la consommation d'oxygène ou la destruction de la matière organique.

Cela donne de grandes possibilités pour la détermination des rélations quantitatives entre la production et la biomasse. En même temps il est très important d'obtenir les caractéristiques physiologiques et d'éclairer l'importance de différentes espèces principales pour la production primaire du plancton. De pareilles études ont été exécutées à la Station Biologique de Sébastopol par N. P. Bessemjanova (1937) qui avait étudié la corrélation entre l'intensité de la photosynthèse, de la biomasse et de la vitesse de la division de quelques espèces des diatomées (*Skeletonema costatum* (Grew) Cl., *Cerafaulina bergonii* Perag., *Cyclotella caspia* Grun., *Leptocylindrus danicus* Cl., et *Nitzschia closterium* W. Sm.).

Parmi les travaux de cet ordre on peut indiquer les études faites par Lanskaja et Sivkov (1950) qui sont aussi faites sur les algues du plancton de la Mer Noire.

S. V. Brouévitch et d'autres auteurs au cours des travaux hydrochimiques se sont largement servis de la méthode du calcul de la production primaire d'après les variations diurnes d'oxygène dissous dans l'eau. On donne souvent la préférence à cette méthode parce qu'elle n'est pas liée avec les conditions artificielles. Il a été proposé plusieurs modes de calcul de la production primaire d'après les observations des variations diurnes d'oxygène dissous. (Brouévitch, 1936, 1937; Winberg et Jarovitsina, 1939; Fedosov, 1955.)

Cette méthode est basée sur la supposition que la vitesse de l'abaissement de la concentration d'oxygène dans la nuit permet de tenir compte de la vitesse d'absorption de l'oxygène durant la journée quand les deux procédés se produisent simultanément.

$$PO_2 = 24 \times \Delta O_2 + (O_2'' - O_2')$$

PO_2 = la valeur totale d'oxygène libéré pendant vingt-quatre heures (la production primaire).

ΔO_2 = l'abaissement de la concentration d'oxygène dans l'eau pendant une heure dans la nuit.

O_2'' et O_2' = la concentration d'oxygène dans l'eau au commencement et à la fin des vingt-quatre heures.

Une des plus simples méthodes parmi celles proposées par S. V. Brouévitch, a été largement em-

ployée. Sur la base de ses propres observations sur la Mer Caspienne il a fait la conclusion que la production d'oxygène de vingt-quatre heures (PO_2) est proportionelle à la différence entre la teneur maximum d'oxygène dans l'eau dans l'aprèsmidi et la teneur minimum d'oxygène le matin (Δ O_2 max.).

D'après S. V. Brouévitch $PO_2 = 1{\cdot}6\ \Delta\ O_2$ max.

A l'aide de cette méthode S. V. Brouévitch (1937) a déterminé la production primaire et le "turnover" du plancton de la Mer Caspienne. La valeur de ce "turnover" d'après S. V. Brouévitch est en moyenne 1·5 par jour ou 300 par an. Ces données ont été utilisées par lui (1939) dans les calculs préliminaires de la production annuelle du plancton de la Mer Caspienne qui, d'après S. V. Brouévitch fait 100,000,000 tonnes du poids sec quand la masse biologique moyenne du plankton est 350,000 tonnes.

Il faut noter que cela fait presque 100 g. C/m².⁄ an.

V. G. Datsko (1955) a déterminé la production primaire du plancton pour chaque mois en utilisant les données de plusieurs années sur les variations diurnes dans la Mer d'Azov aux eaux basses de grande fertilité. A l'aide de ses propres données V. G. Datsko a conclu que 1 ml. O_2 est equivalent à 0·41 mg. C. En utilisant ce coefficient V. G. Datsko a trouvé que la production primaire brute de la Mer d'Azov fait 17×10^6 tonnes C par an.

C'est une valeur considérable parce qu'elle fait 410 g. C/m².⁄ an. Bien que la méthode de calcul de la production primaire d'après les variations de tenue d'oxygène soit utilisée pour des conclusions de grande importance il n'est pas privé de sources considérables d'erreurs. A cause du déplacement des eaux pendant vingt-quatre heures la méthode d'observation des variations diurnes d'oxygène dissous peut donner des résultats sûrs seulement dans le cas où les variations de tenue d'oxygène totale sont examinées dans toute la colonne jusqu'au fond ou jusqu'à la profondeur où les variations diurnes cessent.

Même à cette condition on admet pour toutes les formes de calcul que l'échange d'oxygène entre l'atmosphère et les couches supérieures de l'eau se produit avec la même vitesse pendant la journée et pendant la nuit.

On suppose que la source unique des variations diurnes de tenue d'oxygène dans l'eau n'est que l'interruption de la photosynthèse pendant la nuit. En réalité, dans les bassins aux eaux basses et à grande fertilité du plancton, c'est à dire justement dans les conditions dans lesquelles cette méthode a pu être appliquée (la profondeur moyenne de la Mer d'Azov est 6·8 m.) les conditions différentes de circulation des eaux pendant la journée et pendant la nuit ont une grande signification.

En nous basant sur des exemples concrets des bassins aux eaux basses et avec une haute fertilité du plancton nous avons eu la possibilité de démontrer (Winberg, 1955) que l'interruption de la photosynthèse pendant la nuit dans ces conditions n'est pas la seule cause des variations diurnes de la tenue d'oxygène dans l'eau, et même n'en est pas la principale. En été, le fait que la stabilité verticale des eaux est plus grande dans la journée que pendant la nuit est de grande signification. Grâce à cela la consommation d'oxygène par les sédiments se produit avec une vitesse minime et même peut cesser pendant la journée, tandis que dans la nuit à cause de la circulation des eaux, elle se produit avec une grande vitesse. C'est pourquoi on ne peut pas calculer avec exactitude la vitesse d'absorption de l'oxygène pendant la journée d'après la vitesse de l'abaissement de la tenue de l'oxygène pendant la nuit. Outre cela il faut dire que dans les bassins où à cause d'une photosynthèse très intense les couches supérieures de l'eau sont supersaturées par l'oxygène, comme par exemple sur la Mer d'Azov la différence dans la vitesse de l'échange d'oxygène avec l'atmosphère pendant la journée et pendant la nuit doit avoir une grande importance. Ainsi, que Redfield (1948) l'a démontré pour l'hiver et l'été et pour la même cause pendant la stratification thermique bien marquée, le coefficient d'échange avec l'atmosphère dans la journée doit être plus bas que pendant la circulation thermique nocturne.

Dans les cas où l'eau est supersaturée par l'oxygène, ce facteur de même mène à l'augmentation de la vitesse d'abaissement de tenue d'oxygène, qui est plus grande dans la nuit que dans la journée. Si on ne prend pas en considération de pareils phénomènes on obtient pour la production primaire du plancton des valeurs trop grandes.

Les études spéciales effectuées simultanément à l'aide de deux méthodes: la méthode d'oxygène des bouteilles claires et assombries et celle des observations de changement diurne d'oxygène, ont montré que la production primaire calculée à l'aide des variations d'oxygène diurnes est beaucoup supérieure (même deux fois plus grande) que la production calculée à l'aide des bouteilles claires et assombries (Winberg et Jarovitsina, 1936; Winberg, 1955).

Le même résultat a été obtenu par Dobrjanskaya (1954) dans le travail qu'elle a consacré spécialement à la comparaison des deux méthodes. Les derniers temps les mêmes résultats ont été obtenus sur la Mer Caspienne. Nos considérations ne signifient pas que les calculs à l'aide des variations d'oxygène diurnes ne sont pas valuables. Au contraire, dans les bassins les plus fertiles, quand les variations diurnes de tenue d'oxygène sont bien exprimées, cette méthode est très utile et elle mérite d'être développée. Pourtant il ne faut pas oublier que dans les bassins relativement plus fertiles et aux eaux basses les variations diurnes de tenue d'oxygène dissous ont lieu non seulement à cause de l'interruption de la photosynthèse dans la nuit. C'est pourquoi une analyse exacte de la distribution de la température de la circulation des eaux dans des moments différents du jour et de la nuit permet d'utiliser les observations des variations diurnes d'oxygène dissous dans l'eau, non seulement pour le calcul de la liberation photosynthétique d'oxygène, mais aussi pour la détermination de la vitesse de consommation d'oxygène par le fond et de l'échange avec l'atmosphère.

Les isotopes radioactives en URSS ont été au début appliqués pour l'étude de la production primaire aux eaux douces. S.U. Kusnetsev (1955) plongeait dans le lac des bouteilles claires et assombries remplies d'eau non filtrée et d'eau d'où le phytoplancton était enlevé par la filtration. Dans toutes les bouteilles il a été introduit $Na_2{}^{14}CO_3$. De cette manière il a été prouvé que dans l'eau des lacs le phytoplancton est le producteur principal et que la hemosynthèse bactériale a une signification minime.

Un certain intérêt présente la modification de la méthode Steemann Nielsen, proposé par U. Sorokin (1955, 1956), qui a été élaboré sur les eaux douces à l'Institut de Biologie des lacs artificiels de l'Académie des Sciences de l'URSS et ensuite elle a été appliquée sur le Pacifique à bord du "Vitiaz". S. I. Sorokin reçoit la valeur de la production primaire du plancton sous 1 m². de la manière suivante. En qualité de valeur principale il se sert des résultats des mesures de la vitesse d'assimilation CO_2 à la lumière naturelle, ce qui peut être fait à bord d'un navire à l'aide des bouteilles plongées dans l'eau courante durant une journée ou la moitié d'une journée. Les valeurs reçues de cette manière ne montrent que la vitesse de l'assimilation de CO_2 dans les couches supérieures de l'eau les plus éclairées. Pour connaître combien de CO_2 est assimilé durant une journée sous 1 m². il faut savoir la distribution de la vitesse d'assimilation de CO_2 dans de différentes profondeurs. La vitesse d'assimilation sur les profondeurs données diffère de l'assimilation dans les eaux supérficielles à cause de: 1) différentes conditions de l'éclairage dépendant de la transparence de l'eau dans la région donnée; 2) la distribution irrégulière du plancton.

Pour savoir comment l'assimilation de CO_2 change dans la région donnée et dans des conditions météorologiques déterminées sous l'influence des conditions d'éclairage il suffit de remplir des bouteilles avec de l'eau prise dans un seul horizon et les placer dans de différentes profondeurs pour la même période. Les résultats des mesures d'assimilation à la profondeur h (C'_h) peuvent être rapportés à l'assimilation mesurée aux mêmes conditions près de la surface (C'_0) à l'aide d'un coefficient,

$$C'_h = K_t \, C'_0$$

La distribution des coefficients K_t d'après les profondeurs montre la dépendance de la vitesse relative de l'assimilation de CO_2 de la lumière dans les conditions données. Pour connaître comment la distribution irrégulière du plancton agit sur la vitesse d'assimilation il suffit de prendre de l'eau des profondeurs différentes et de déterminer la vitesse d'assimilation de CO_2 dans les mêmes conditions, par exemple à bord d'un navire à l'éclairage naturel. Les valeurs reçues (C''_h) peuvent se rapporter à l'aide d'un coefficient à l'assimilation du plancton de l'eau mesurée aux mêmes conditions, qui a servi pour la détermination de la valeur principale (C'_0),

$$C''_h = K_z \, C'_0$$

En réalité la vitesse d'assimilation à la profondeur h (C_h) dépend de deux facteurs et se rapporte avec la vitesse d'assimilation à l'éclairage complet (C_0) de la manière suivante:

$$C_h = C_0 \times K_t \times K_z$$

A l'aide de cette méthode, quand la valeur C_0 est mesurée et les coefficients K_t et K_z connus, la valeur C_h peut être calculée. C'est à dire, les vitesses de l'assimilation de CO_2 peuvent être exprimées par la courve qui montre la distribution de la vitesse d'assimilation de CO_2 aux profondeurs. Ayant cette courve il n'est pas difficile d'obtenir la valeur de l'assimiliation de CO_2 sous 1 m².

La comparaison de la distribution de l'assimiliation de CO_2 aux profondeurs calculées au moyen de cette méthode avec les résultats des mesures directes de la vitesse de l'assimiliation de CO_2 aux profondeurs différentes a montré la coincidance des données.

Le mérite de la méthode de Sorokin consiste en ce qu'elle est presque libre des admissions conventionelles et qu'à l'aide de cette méthode on peut prendre compte des influences non seulement des conditions de lumière, mais aussi de la distribution irrégulière du plancton dans la direction verticale, ce qui a une grande signification comme les données obtenues l'ont démontré. Il est important que la détermination des coefficients K_z puisse être faite à bord d'un navire.

On n'a pas besoin d'obtenir les coefficients K_t à chaque station car l'influence des conditions de lumière sur l'assimiliation de CO_2 dépend de la transparence de l'eau, qui change peu dans de larges régions. Néanmoins au passage dans la région avec d'autres conditions hydrologiques il faut déterminer les coefficients K_t ainsi que pour chaque période des observations. L'application de cette méthode sur le Pacifique au cours du trajet du "Vitiaz" a donné des résultats qui ont montré des différences charactéristiques des régions différentes en ce qui concerne la valeur de la production primaire du plancton sur 1 m². et la distribution de la vitesse d'assimiliation de CO_2 d'après les profondeurs.

Ayant examiné certaines méthodes de la mesure de la production primaire du plancton, qui ont été appliquées aux études exécutées en URSS, il faut conclure, que chaque méthode peut être utile et même indispensable dans certaines conditions et ne peut pas être utilisée dans d'autres. Une étude de la production primaire du plancton menée avec succès ne peut se produire qu'à l'aide d'une utilisation critique de toutes les méthodes connues.

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C. 1.

Carbon Production in the Sea at the Smith's Knoll Lightvessel

By R. S. WIMPENNY

The ^{14}C method

The production of carbon in the sea near the Smith's Knoll lightvessel has been measured using the ^{14}C methods of STEEMANN NIELSEN (4 & 5) (a) by the exposure of samples dosed with a known activity in an illuminated tank or (b) by bottles floating in the sea throughout a day and containing water from the levels at which they were floated. I am indebted to Professor STEEMANN NIELSEN for arranging to have the activities counted at his laboratory in Copenhagen.

The observations which were made in February and April and for three different Octobers are given in Table 1. The length of exposure of the samples to ^{14}C was never more than a day so that the amount of ^{14}C synthesized and subsequently lost as respired CO_2 is thought likely to have been small (3). Nevertheless it must be remembered that the values obtained are likely to be slightly less than the true amount of carbon photosynthesized. The figures in the table vary from 112 to 2·5 mg. of C under a square metre a day and are much lower than other similar observations in coastal areas. They more nearly resemble STEEMANN NIELSEN's oligotrophic areas and some of the values he obtained near river mouths.

The lowest production was in February and two of the three October figures were similar to April while the other was much higher. Substantial blooms of large diatoms are frequently found in the neighbourhood of the Smith's Knoll lightship in October and the indications of such plankton samples as have been taken in this area are that the autumn flowering is at least as important in this locality as that of the spring. We should not be surprised, therefore, if carbon assimilation is found to be at its height in this month.

The penetration of the light passed through a V.G. 9 Schott green filter was measured on several occasions when ^{14}C observations were made and a series was also taken near this lightship in June 1939. These observations appear in Table 2 from which it appears that water is generally rather turbid being clearest in June. On a dull February day, however, the light was cut to 1 % at from 2 to 4 metres, the turbidity altering during the same day, possibly as a result of the changing strength of the tidal flow and its contained detritus.

Table 1

Production of organic carbon according to the ^{14}C method at Smith's Knoll lightvessel

Date	23.X.53	18.II.54	18.II.54	28.X.54	21.IV.55	13.X.55
Type of Experiment	Illuminated tank	Illuminated tank	Floating bottles	Illuminated tank	Floating bottles	Illuminated tank
Duration	3 hrs.	3 hrs.	10 hrs. 10 mins.	3 hrs.	$14^{3}/_{4}$ hrs.	3 hrs.
Temperature, °C.	13·5	5	3·24—3·36	11—12	4·77—4·78	13—14·5
Depth of 1 % of surface green light	15 m.	3	3	11	—	20 m.
Production of C under m.² per day in mg.	112	6	2·5	26·9	25	17

Table 2

The percentage penetration of green light into the sea referred to sea surface at the Smith's Knoll lightvessel

Station 52, Cruise III, "Sir Lancelot", 18. February 1954

Time	0735	0908	0958	1106	1150
	0750	0914	1002	1110	1159
Depth					
0	66	44	40·3	39·75	38·79
1	—	—	—	—	11·02
2	—	—	—	—	2·56
2½	6·7	4·04	3·18	0·45	0·40
3	—	—	—	—	0·43
4	—	—	—	—	0·13
5	—	0·36	0·14	0·03	—

Time	1203	1410	1505	1628
	1208	1419	1514	1637
Depth				
0	60·71	17·0	14·37	22·33
1	7·0	5·59	2·45	3·42
2	3·03	0·66	0·08	1·71
3	0·43	0·31	0·14	0·58
4	0·20	0·21	0·08	0·27
5	0·04	0·05	0·03	—

Station 55, Cruise J., "George Bligh", 26. June 1939

Time	0408	0518	0911	1315	1738
	0420	0526	0917	1320	1745
Depth					
0	67·5	60·1	85·04	84·1	57·45
5	35·0	33·3	50·39	24·5	26·09
10	17·0	22·5	24·68	11·61	10·87
15	5·90	6·29	11·63	5·81	5·11
20	2·41	3·34	7·55	4·17	2·17
25	0·78	1·80	2·22	2·31	1·19
30	—	1·00	0·74	1·09	0·49

Station 104, Cruise XVI, "Sir Lancelot", 23. October 1953

Time	0610	0700	0810	0907	1001
	0620	0708	0822	0916	1009
Depth					
0	78·6	35·1	55·6	53·0	59·8
5	15·6	7·7	25·0	18·7	23·5
10	3·3	0·3	4·4	2·1	1·5
12½	—	0·06	3·2	—	—
15	—	—	0·52	0·18	0·5
17½	—	—	0·14	—	—
20	—	—	0·05	0·01	0·02

Time	1100	1154
	1115	1208
Depth		
0	50·0	56·7
5	32·7	40·3
10	7·1	11·04
15	1·02	3·13
20	0·2	0·46
22½	0·1	—
25	0·04	0·14
27½	0·02	0·09
30	—	0·03

Station 42, Cruise XVIII, "Sir Lancelot", 28. October 1954

Time	1018	1150
	1032	1200
Depth		
0	58·34	57·24
5	9·32	10·18
10	0·9	1·74
12	0·1	0·28
14	—	0·01

Station 31, Cruise XV, "Sir Lancelot", 13. October 1955

Time	1244
	1251
Depth	
0	94·0
1	64·81
2	48·13
3	30·0
4	31·70
5	27·0
10	16·03
15	6·7

The oxygen method

Big differences have been found between the ^{14}C and oxygen methods of estimating carbon production in oligotrophic waters but in the eutrophic waters they are stated to have given similar results (3). The reason for this difference is still a matter under discussion elsewhere.

In this place, however, I would point out that the low figures given by the ^{14}C method in the Smith's Knoll area might make it resemble the oligotrophic rather than the typical coastal area and that for this reason comparable oxygen estimates might give a discrepancy. Unfortunately there are no strictly comparable oxygen method estimates available but there are some results for 26. June 1939 when covered and uncovered bottles were drifted at the surface through a day of 17 hours 27 minutes. The results expressed in ml. per litre were as follows:—

Original oxygen in sea	Exposed bottle	Covered bottle
5·67	5·98	5·41

The nett production (exposed bottle minus original) is equivalent to 0·17 mg. of carbon per litre, or estimating to a depth of 30 m., 5·1 g. of carbon under a square metre compared with ·112 g. under a square metre for the highest value obtained by the ^{14}C method.

Photosynthetic rate

The October 1953 observations occurred in water occupied by diatoms including a big species, the patch-forming *Rhizosolenia styliformis*. A sediment sample count at 10 m. using volumes given by Savage (7) for most of the species and one computed by myself for *Rhizosolenia styliformis* permitted an estimate of the standing crop volume of this phytoplankton. Allowing a fifth of this for dry matter (1 and 2) converting this to dry organic matter by subtracting 25 % for ash and then taking 44 % of the residue as carbon (6), the standing crop of carbon in the phytoplankton was estimated as 45·4 mg. per m^3., most of which would be based on the *Rhizosolenia styliformis* cells. Relating this to the daily uptake of carbon of 13·8 mg. at 10 m. we have a daily photosynthetic rate of 30·6 %.

Carbon uptake at three different temperatures was investigated in March 1957 using surface water taken not far from the Smith's Knoll lightship. The results given in Table 3 show that the uptake per hour roughly doubled between 5 and 10° C. but increased more modestly from 10 to 15° C. Related to the carbon estimated from a count of the standing crop, these uptakes gave daily photosynthetic rates of 55·2, 122·5, and 168 % at 5, 10, and 15° C. respectively.

Table 3

Water collected from the surface over the Eastern Deep water on 22.3.56 and illuminated at 5°, 10°, 15° C. at 16,000 lux for three hours

	Uptake of C in mg. per hours using ^{14}C
Experiment at 15°	1·25
Mean	0·807
	1·028
Experiment at 10°	0·803
Mean	0·697
	0·750
Experiment at 5°	0·350
Mean	0·327
	0·338
Standing crop of phytoplankton from counts as mg. C per m^3	6·12
% daily increase at 15°	168
% daily increase at 10°	122·5
% daily increase at 5°	55·2

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C. 2.

Some Experiments Using the ^{14}C Technique

By D. H. Cushing

Between March and June 1954 some estimates of production were made in the area between the Devil's Hole and Flamborough Head. The same observations were used to make an estimate of the algal reproductive rate; at the same time two short grazing experiments were carried out. An experiment subsequently carried out in the laboratory is also described.

Method

The main object of the ^{14}C work was to obtain an estimate of the algal reproductive rate. This was estimated from measures of algal volume (converted to carbon) at four or five depths combined with measurements of carbon production made at the light intensities corresponding to those found at those depths. As the work was carried out in an experimental tank on the ship, the light intensities at those depths were simulated with the use of neutral density filters and a battery of incandescent lamps in series with a variable resistance. Perspex bottles of rectangular section were used; three sides were blackened and the fourth was covered with two filters, one representing the colour of the sea water (Chance, ON 20 roughly represents Jerlov's, 1951, Coastal type I) and the other a neutral density filter. An experiment showed that the bottles blackened on three sides yielded results that were 29 % higher than fully clear bottles (16 observations over a full range of light intensities); this result cannot be explained although it is pointed out that in the sea it will be darker below than above a given diatom.

Light measurements were made with a barrier relay cell to obtain the percentage light transmission at the depths of sampling. Measurements of light intensity as kilolux were made with a calibrated cell and a green Schott filter (VG 1); all light measurements were made at midday. The duration of daylight for particular intensity bands (e.g., 5—10 %) was calculated, so the production of carbon at any particular depth was corrected for the length of day.

Measurements of carbon production were made by Steemann Nielsen's (1952) method. Phytoplankton was counted by the sedimentation technique and raised to algal volume with size measurements. The algal volume, as μ^3/litre, was converted to carbon using the ratio $\frac{\text{algal volume}}{\text{carbon}} = 11$.

Results

1. Reproductive rate

The rates of carbon production as mg. C/m³./3 hrs. were obtained at five depths (light intensities of, for example, 50 %, 33 %, 25 %, 10 %, and 5 % of that at the surface). At each of these five depths estimates of carbon as standing stock were made from measures of algal volume (in μ^3/litre). The rate at one depth as mg. C/m³./day was expressed as a percentage of the standing stock as mg. C/m³. The results are shown in Table 1, as averages for the whole photic zone.

Table 1. Algal reproductive rates in the North Sea in April and May, 1954

Date	Standing stock in mg. C/m.³	Daily production in mg. C/m.³/day	% production/day
8.—15. April...	16·6	3·6	21·7
24.—28. April...	37·5	10·7	28·7
4.—14. May ...	12·4	7·6	61·3
23.—26. May ...	16·0	13·2	82·5

From the cell size decrease method for diatoms only in the spring of 1949 (Cushing, 1955), the following estimates of algal reproductive rates were made:—

10.-30.IV.1949 38 %/day
23.IV.—16.V.1949 22 %/day

The latter method includes the effects of vertical turbulence, whereas with the carbon method, the reproductive rate is that in the photic zone, as if

there were no turbulence; the average for the carbon method is 48·5 %, whereas that for the cell size decrease method is 30 %, for roughly the same period.

2. Total production

Surface observations and observations at the 1 % level of light intensity were not taken. When however the observations at other levels of light intensity were plotted on semilogarithmic paper, with the percentage light intensity on the logarithmic ordinate, the production rate at the surface and at the 1 % level were estimated by extrapolation; as no thermocline had developed this was considered to be a reasonable procedure as it was assumed that a steady vertical mixing was still taking place to some extent. The measurements of carbon production and the extrapolated estimated were plotted against depth and the total production in g. C beneath 1 m². was estimated by measuring the area beneath the curve. The results are given in Table 2.

Table 2. Production as g. C/m².day off the north-east coast of England in April and May, 1954

Cruise	Station	Production	Depth of photic zone m.
VI Sir Lancelot 1954.	34	0·138	39
VIII "	14	0·151	39
IX "	9	0·415	45
IX "	33	0·372	37

Mean 0·219 g. C/m².day.

The stations were not necessarily made at the points of highest standing stock, but were made when the ship was steaming steadily with a following wind. Steemann Nielsen (1954) made four measurements in the "Galathea" in May on approaching the English Channel at Stations 769—775; their mean was 0·32 g.C/m².

3. Grazing

Two experiments were made on the grazing activity of *Calanus*, stage V. In each experiment, an estimate of algal reproductive rate was made as a control and one or two *Calanus*, stage V, were put into an 140 ml. bottle. The experiment was run normally, except that the animal was picked out immediately it appeared in the filter funnel; it was not allowed to fall on to the membrane filter.

The results of the two experiments are given in Table 3.

Table 3

	Depth m.	% light	Light intensity kilolux	Standing stock mg. C/m.³	Production mg.C/ m.³/3hrs.	Animals
Expt. I	7·5	33 %	26·6	13·7	3·94 2·03	2 *Calanus* V
Expt. II	9·5	25 %	20·0	25·54	3·58 2·60	1 *Calanus V*

Let Q be the standing stock in mg.C/m³.,
let q be the quantity produced in mg.C/m³./3 hrs.,
$q = Q\,(e^{Rt} - 1)$ where R is the algal reproductive rate during time t.

When a grazer is introduced,

$q = Q \quad (e^{(R-G)t} - 1)$ where G is the algal mortality rate due to grazing during time t.

The fraction taken by grazing, S, is

$$\frac{G}{R-G}\left(e^{(R-G)t} - 1\right)$$

The estimates are:—

	Standing stock mg. C/m³.	R	G	S	S/animal
Expt. I	13·7	0·255	0·115	1·69	0·121 μg. C
Expt. II	25·54	0·14	0·045	1·21	0·17 μg. C

The average quantity taken per animal was 0·14 μg. C. The average displacement weight of *Calanus* V is 0·59 mg. (Riley, Stommel, and Bumpus, 1949). Using a displacement weight/dry weight ratio of 9:1 (Deevey, 1952) we have for one *Calanus* V:—

Displacement weight	Dry weight	Carbon
0·59 mg.	0·07 mg.	0·033 mg. (33 μg. C)

The quantity of carbon grazed by each animal per day was 1·12 μg. which is 3·4 % of the animal's carbon content.

The volume swept clear, F, is defined:— $F = VG$, where V is the volume of culture medium/animal.

For Expts. I and II, $F = 69$ and 54 ml./day respectively. It is possible that this is not the maximum potential grazing effort because the volume of the experimental vessel was only 140 ml.; however, the animals were feeding at a rate sufficient to support them (from the oxygen experiments of Marshall and Orr (1935), *Calanus* needs 3—7 % of its dry weight per day as a maintenance ration).

4. Photosynthesis and phosphorus content

A culture of *Biddulphia regia* in phosphorus-free sea water was used; the medium was made by exhausting the nutrients with algae and then by adding N, Mn, and Fe to excess. Six bottles (of 150 ml. capacity each) were run with three ampoules each for three hours; in each bottle were 100 cells of *Biddulphia regia* and varying quantities of phosphorus were added.

Table 4. Relation between photosynthesis and phosphorus content

Bottle	mg. P/m³.	Counts per minute above background
1	0	50·1
2	2	48·8
3	4	45·2
4	6	36·5
5	9	42·4
6	10	50·6

The cells had been kept for 24 hours in phosphorus-free sea water before the experiment started and then were picked out and placed in the culture bottles with fresh phosphorus-free sea water; it is possible that some phosphorus was left in the cells as a storage product. It is possible that the phosphorous content of the medium has no immediate effect on the photochemical processes.

Discussion

In addition to the estimates of production as g.C/m².day made with STEEMANN NIELSEN's (1952) method, the ^{14}C technique has been used to make estimates of the algal reproductive rate and the grazing rate of *Calanus*, stage V. A preliminary experiment on the relation between photosynthesis and phosphorous content is described.

The estimate of reproductive rate depends on a firm estimate of the algal volume/carbon ratio. This is discussed in another paper presented to this meeting; the one used in this paper is that reported in CUSHING (1957) before the experimental work on the ratio was started.

The estimate of grazing rate is a very preliminary one really to show how the results may be manipulated in order to compare the "volume swept clear" with the quantity eaten by each animal.

Both types of experiment rest on the assumption that the weight of carbon built into the algal cell during a ^{14}C experiment is proportional to the reproductive rate, i.e., that ^{14}C measures photosynthesis -respiration.

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C. 3.

Measurements of Primary Production in the Western Barents Sea

By John Corlett

During July 1953 and May 1954 a few measurements of primary production were made from the research vessel "Ernest Holt" using the ^{14}C method of Steemann Nielsen (1952). The 1953 measurements of ^{14}C were inaccurate because the quantities were small and unskilful handling introduced large errors; but several good series of measurements of submarine light penetration were made. In 1954 the ^{14}C measurements were satisfactory but no underwater light measurements could be made, and so the extinction coefficients obtained in 1953 were used in the 1954 calculations.

Light penetration

Measurements of the penetration of green light were made with two Weston photoelectric cells which had been calibrated at the National Physical Laboratory. For the calibration and during the measurements each cell was covered by a diffusing window and a green filter with maximum transmission at 5300 Å (Chance's 0 gr. l). This filter has an "integrated visible transmission" of 22 %. The measurements were made from the after hydrographic laboratory of the "Ernest Holt". The "deck cell" was mounted in gimbals on the roof of the laboratory and the submarine cell in a watertight brass case was lowered over the stern from the hydrographic winch. Measurements were made with the submarine cell "just above the water", at 0·5 metres and then at 5 metre intervals until 70 or 75 metres of wire was out; the wire angle was measured at each depth and the deck cell was read, through the same galvanometer, immediately before each reading of the submarine cell. The extinction coefficient, K, has been calculated from the formula:—

$$I_L = I_0 e^{-KL}$$

where I_0 is the surface illumination and I_L the illumination at depth L. In Table 1, two values of K are given, one with I_0 as the illumination just above the surface and L as the depth of 1 % of the above surface illumination, and the second with I_0 as the illumination at 0·5 metres and L as the maximum depth of observations.

The first five measurements in Table 1 were made in arctic water of the Bear Island Current on the Spitsbergen Shelf, and the last one was in mixed Atlantic and arctic water in the Hope Island Deep.

Table 1. Penetration of green light in the western Barents Sea

Position N.	Position E.	Date 1953	Local (sun) time	Sky	Surface light (green) (lux)	Deepest record, m.	Depth of 1 % surface light, m.	Extinction coeff. (K) Surface to 1 % point	Extinction coeff. (K) 0·5 m. to max. depth
74°50′	25°03′	14/7	2314—2330	overcast	2,300— 2,750	75	70	0·066	0·058
74°57′	24°32′	15/7	1013—1034	,,	15,500—19,000	57·3	66·5 ex[1])	0·069	0·063
,,	,,	,,	1415—1430	,,	11,550—12,450	67·6	64	0·072	0·065
,,	,,	,,	1849—1902	,,	6,850— 7,400	45	63 ex[1])	0·073	0·062
74°57′	24°29′	16/7	1146—1205	clear	41,600—43,600	61·4	60·5	0·076	0·074
74°50′	27°43′	,,	1916—1927	,,	21,300—23,500	70·5	47	0·098	0·080

[1]) ex indicates "extrapolated".

Production measurements

Measurements of primary production were made at three stations in May 1954 by "Method II" of Steemann Nielsen (1952). Samples of water from 0, 5, 10, 20, 30, and 50 metres were put into 250 cc. glass bottles and 1cc. of active $NaHCO_3$ solution added to each. (The ampoules of active solution were supplied from Professor Steemann Nielsen's laboratory, and each contained about 3 microcuries of activity). The bottles were rotated in a water-bath for 3 hours at nearly constant temperature, with illumination of 11,000 lux. The samples were then filtered through collodion membranes and dried. Professor Steemann Nielsen kindly counted the activity of the membranes in his laboratory and calculated the production from the counts. He says "the computation of productivity per m².day is done in the same way as during the "Dana" expedition to north-west Greenland 1954. A temperature correction is made (5 % per ° C.)". The extinction coefficient of 0·07 was taken from the 1953 work.

The results are as follows:—

Station 39
Position 75°15′N., 25°00′E. 11. May 1954.
Temperature in tank 5·5 °C.

Depth, m.	Temperature in the sea °C.	Production mg. C/m.³/hour
0.....	—0·86	1·7
5.....	—0·63	2·0
10.....	—1·42	2·6
20.....	—1·56	8·6
30.....	—1·66	7·8
50.....	—1·50	7·7

The calculated production is:—

$$\frac{(3{\cdot}4+4{\cdot}0+5{\cdot}2+17{\cdot}2+7{\cdot}8+7{\cdot}7)\times 21\times 40\times 0{\cdot}7}{10\times 2}$$

= 1331 mg. C/m.²/day
= 1·33 g. C/m.²/day

Where 21 is the number of hours from sunrise to sunset, 0·7 is the temperature correction, and 40 is the depth of the photosynthetic layer in metres. Steemann Nielsen says that "66 m. as indicated by the extinction coefficient is presumably too much at this station".

Station 63
Position 74°25′N., 14°34′E. 15. May 1954.
Temperature in tank 7°C.

Depth, m.	Temperature in the sea °C.	Production mg. C/m.³/hour
0.....	5·66	0·51
5.....	5·65	0·40
10.....	5·62	0·38
20.....	5·60	0·71
30.....	5·49	0·41
50.....	5·34	0·49

The calculated production is:—

$$\frac{(1{\cdot}02+0{\cdot}80+0{\cdot}76+1{\cdot}42+0{\cdot}41+0{\cdot}49)\times 21\times 66\times 0{\cdot}95}{10\times 2}$$

= 322 mg. C/m.²/day
= 0·32 g. C/m.²/day.

Station 85
Position 73°53′N., 19°24′E. 18. May 1954.
Temperature in tank 7°C.

Depth, m.	Temperature in the sea °C.	Production mg. C/m.³/hour
0.....	5·36	0·39
5.....	5·26	0·41
10.....	5·26	0·32
20.....	5·24	0·48
30.....	5·12	0·51
50.....	4·81	0·72

The calculated production is:—

$$\frac{(0{\cdot}78+0{\cdot}82+0{\cdot}64+0{\cdot}96+0{\cdot}51+0{\cdot}72)\times 21\times 66\times 0{\cdot}9}{10\times 2}$$

= 276 mg. C/m.²/day
= 0·28 g. C/m².day.

Of these three stations, 39 is in arctic water of the Bear Island Current east of Bear Island, 63 is in the Atlantic water of the West Spitsbergen Current west of Bear Island and 85 is in Atlantic water south of Bear Island. Thus production is highest at this time in the cold arctic water on the Spitsbergen Shelf.

In a paper presented to the Plankton Committee of ICES in 1955, P. T. Marshall discussed the conditions for phytoplankton production in the Bear Island area in terms of the depth of the homogenous layer and the "critical depth" of Sverdrup (1953) and an algal reproductive rate calculated from the light energy in the euphotic zone. He showed that phytoplankton production might be expected to start in the cold water on the bank in March, or as soon after that as the water is ice free, but production in the deeper Atlantic water would not start until May or June. The three ^{14}C experiments in May 1954 fit in with this pattern. Although the temperature records of Station 39 on the bank do not show any obvious thermocline, the depth of the bottom in 165 metres is about the same as the average critical depth for this date.

Water samples taken at the same time as those for the ^{14}C experiments were examined under a reverse microscope and Marshall used the counts at Station 39 to compare with the ^{14}C results. The number of diatom cells under 1 m². was $6{\cdot}23\times10^9$, of which the main constituents were *Thalassiosira gravida* 40 %, *T. hyalina* 24 % and *Fragillaria islandica* 20 %. Using a figure for cell volume of 12,000 μ from Cushing (1955) and the conversion factor of 0·027 from Sverdrup *et al.* (1942) this becomes 2·02 g. C/m². The algal reproductive rate at this date from his own calculations is 0·63 and this gives a figure for plant

production of 1·27 g. C/m².day—which is remarkably close to the 1·33 obtained from the ^{14}C experiments, considering the possible errors in both calculations.

Ryther (1956) presents "a preliminary method for estimating absolute daily photosynthesis beneath a square metre of sea surface from measurements of (1) photosynthesis of a sample of the population at light saturation, (2) the extinction coefficient of light in the water and (3) the daily surface radiation". His formula is:—

$$P = \frac{R_s}{K} \times P_{sat.}$$

where P is the total photosynthesis in mg. C/m².day,
R_s is the relative photosynthesis within the euphotic zone for the appropriate value of total daily surface radiation (s) and is taken from Figure 6 in his paper,
$P_{sat.}$ is the measured total or "gross" photosynthesis at light saturation of a sample of the population, as mg. C/m³./hour,
K is the extinction coefficient.

A calculation of P by this method can be made for the three stations 39, 63, and 85. From Marshall's calculations surface radiation in mid-May in the Bear Island area is about 300 g. cal./cm².day and from Ryther's figure this gives $R_s = 18$. K is put at 0·07 (the value for green light) from the 1953 experiments, and $P_{sat.}$ is the value from the ^{14}C experiments, calculated by Professor Steemann Nielsen, since 11,000 lux is about light saturation for these diatoms.

Thus for Station 39:

$$P = \frac{18}{0{\cdot}07} \times \frac{(3{\cdot}4 + 4{\cdot}0 + 5{\cdot}2 + 17{\cdot}2 + 7{\cdot}8 + 7{\cdot}7)}{10}$$

$= 1{\cdot}16$ g. C/m.²/day,

which is near to Steemann Nielsen's calculation of 1·33 and Marshall's of 1·27.

For Station 63 Ryther's method gives $P = 0{\cdot}13$ compared with Steemann Nielsen's 0·32 and for Station 85, 0·11 compared with 0·28.

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C. 4.

Production Studies in the Northern North Sea

By J. H. Steele

Introduction

Because conditions in the sea can be so variable it is often difficult to decide on the usefulness of single estimates of production made by such methods as the ^{14}C technique (Steemann Nielsen, 1952). If, however, these measurements could be interpreted in terms of the environment then it would be possible to explain the effects of changing physical conditions. This is an ideal which cannot be attained because of the complexity of possible environmental factors but, by making the attempt, some understanding can be found for the variations in production estimates.

In particular it has seemed wortwhile to investigate the quantitative relations between some comparatively simple hypotheses. The usual factors considered have been light, transparency, vertical mixing, and nutrient deficiency; also two "biological" factors, sinking of plants and their grazing of zooplankton. One of the main problems has concerned the way in which nutrients control production and for this phosphate data has been used most often because of the comparative ease of the chemical analyses. The study of such data can provide not only estimates of production but also an interpretation of the values found.

Beyond this, attempts to explain the production cycle lead to mathematical models of the causal relations supposed to operate. This approach which is due mainly to G. A. Riley has been sufficiently successful to make further development worth trying.

In this paper I will describe the basic problems of the "phosphate method" and give some results of its use in the northern North Sea. I will also outline briefly a mathematical model relevant to the same area and give some detailed comparisons of the model, the phosphate results and the ^{14}C technique.

The phosphate method

Changes in phosphate concentration in the sea are caused by uptake and release of the nutrient by plants and animals, but also by water movement. Thus before the biological effects can be found the variations due to the changing water must be determined.

Because of its effect on production, the most important form of water change is vertical mixing; when this is the only significant type of motion it can be estimated from a study of the changes in vertical temperature profiles. Thus, for the northern North Sea, the first area chosen for study was the Fladen Ground where lateral changes were expected to be unimportant (Steele, 1956).

By sampling for temperature and phosphate on as many occasions as possible, the productive season was divided into fairly short intervals. The effects of vertical mixing were subtracted from the total observed phosphate changes to give for each interval an estimate of the "biological change". These changes showed a decrease in the surface layer (surface to 30—50 m.) and an increase below. The problem then was to relate the quantities of phosphate removed from the surface layer to the amounts of plant organic matter produced. The simplest way of doing this was to assume that the ratio of phosphorus to carbon found in plants (Sverdrup, Johnson, and Fleming, 1942, p. 237) can be used to provide a conversion factor which thus gives an estimate of production in terms of carbon.

This approach has many obvious drawbacks. These are the hydrographic problems concerned with the assumption that vertical mixing is the only significant factor and that its effect can be estimated by a simple formula. But beyond this the main difficulties arise from doubts about the role of phosphorus in the metabolism of plankton. These problems are most serious during the summer when nutrient concentrations are low and small amounts of vertical mixing lead to very low values for production. Such results would be fallacious if there was a large regeneration rate for phosphate in the upper layers and this supply was used immediately by the plants. The results would also be too low if the plants used organic phosphorus as has been suggested by experimental results (Chu, 1946; Harvey, 1953).

To study these difficulties ^{14}C estimates were obtained on the Fladen Ground during the summers of 1955 and 1956. The values for production rate given by these data (STEELE, 1957a) compared well with the phosphate estimates, both giving low values of about 0·2 g. carbon per day below one square metre. The 1956 value is shown in Figure 3.

These results help to justify the phosphate method but it is still true that the number of steps required to reach a value for production leave the method open to doubt. Thus, although it can be used to cover a large area, it needs the detailed checks provided by the ^{14}C technique. On the other hand, the various steps in calculation of production also constitute the advantage of this approach. They show the ways in which nutrient deficiency and vertical mixing determine the extent of production during each time interval, and by this means values for production rates are related to the physical environment. For example it was possible to show quantitatively with the Fladen Ground data the effects of variable vertical mixing and the critical nature of high winds at certain periods (STEELE, 1956).

At the same time as the Fladen data were collected, samples were taken at some relatively inshore stations and although the results were more variable the method seemed to give adequate and reasonable estimates of production. For this reason it was decided to survey a fairly wide area of the northern North Sea and to obtain values of production for spring, summer, and autumn. Because of the many uncertainties which could be expected from such results it was thought worthwhile to provide a check by treating the whole problem theoretically. A rather hypothetical study of some relevant aspects of the hydrography of the area has already been written (STEELE, 1957b) and a full account of the mathematical treatment of the production cycle together with the three years' observations will be given elsewhere. The following is a brief description of the general results and of the problems involved.

The mathematical approach

There is such a wide range of possibly relevant factors that any selection for a mathematical model may appear arbitrary. Further, the selection will be biased since the factors chosen must be those which can be expressed in a mathematical form. Yet the models which have been constructed (FLEMING, 1939; RILEY, 1946, 1947; RILEY, STOMMEL, and BUMPUS, 1949; STEELE, 1956) have shown a moderate degree of success. These models have been concerned with the interrelations of observed data for particular cycles and have used estimates of the effects of light, nutrient deficiency, sinking, grazing, and vertical mixing.

One disadvantage of these models is that all the factors are varying throughout the year and it is difficult to disentangle their individual effects. For this reason it was decided to construct a completely theoretical model in which each factor could be varied in turn. For the application to the northern North Sea, the variations in vertical mixing are of most interest and so it is the only variable which will be considered here.

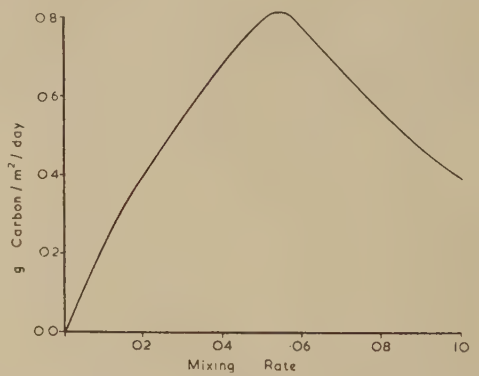

Figure 1. Production rates with varying mixing rate under "steady state" conditions.

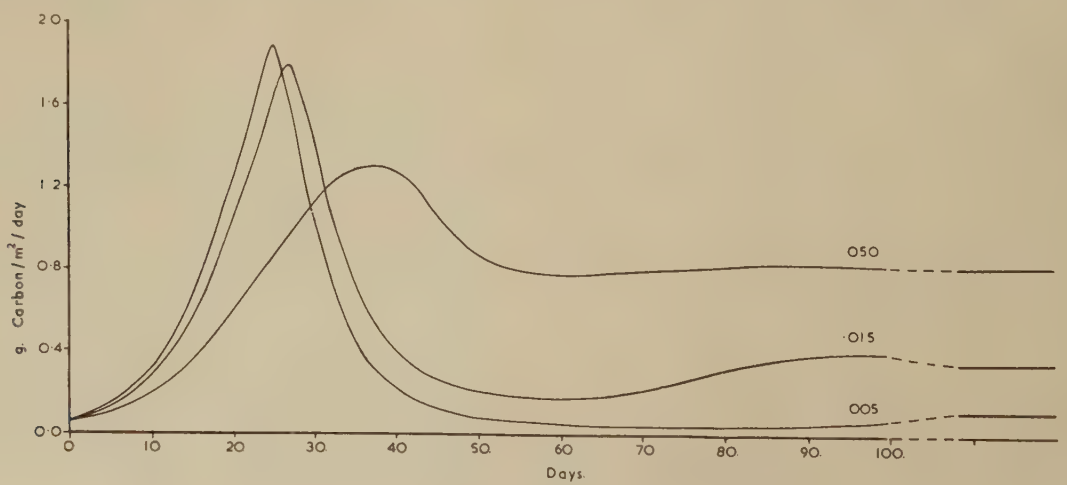

Figure 2. Production cycles for three mixing rates.

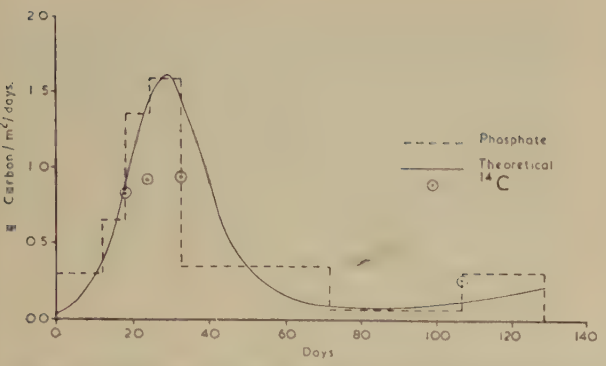

Figure 3. Estimates of production for Fladen during 1956.

The stratification due to the thermocline is idealized into a two-layered sea with mixing between the layers. It is assumed that the upper layer corresponds to the euphotic zone and variations in light and transparency are ignored. The optimum production rate is taken from ^{14}C experiments in early spring and the later effect of nutrient deficiency is introduced by a factor which gives a linear decrease below a phosphate concentration of 0·40 μg. at./l. Grazing and sinking rates are taken from Fladen data (Steele, 1956, 1957a).

On this basis equations can be written down for the changes in phosphate and plant carbon. The latter, however, depends on a measure of the herbivores which are grazing on the plants and an equation for this factor is also necessary. This in turn would depend on carnivorous grazing and, in order to end the series of equations at the herbivore level, an empirical equation was constructed for the zooplankton. It was assumed that the grazing on the herbivores was proportional to the quantity of herbivores and constants for this equations were fitted from the Fladen data for 1953.

In this way three equations were set up for three unknowns—phosphate, plant carbon, and zooplankton carbon. From any solution of these the rate of production can be deduced and in this brief outline it is the production curves that will be considered.

The first use that can be made of this model is to solve it for "steady state" conditions—conditions that will be most nearly approached in high summer. In Figure 1 the values of production rate are graphed against mixing rate (the units for mixing are arbitrary). It can be seen that there is a maximum production rate so that too great mixing tends to inhibit production in the summer.

Turning to changes with time, it can be assumed that production starts at the end of the winter, when the thermocline is first formed. The ensuing cycle can then be calculated for any mixing rate. In Figure 2 the graphs are shown for a low mixing rate, for the optimum "steady" rate and for an intermediate value. It can be seen that after the spring outburst the rates do not tend evenly towards the steady states; it is likely that this feature is a mathematical artifact and it illustrates the shortcomings of a simplified model.

These graphs provide estimates of the expected production during spring and summer for various mixing rates and one conclusion that can be drawn from them is that the higher mixing rates produce a slower outburst with a smaller maximum production rate. To provide a detailed comparison of such graph with observed data, the cycle on Fladen in 1956 has been chosen since both ^{14}C and phosphate values are available. The results are given in Figure 3 where the appropriate rates of mixing were used for the model. There is good agreement between the model and the phosphate estimates: two of the ^{14}C values in spring are lower than the other estimates but this may be explained by the fact that light conditions on the sampling dates were below average.

The autumn outburst

As a further example of the use of the model it will be applied to the problems raised by the autumn outburst. If it were assumed that the simple fact of the breakdown of the thermocline in the autumn caused an outburst by bringing nutrients into the surface waters, then there would always be a production peak in the autumn. In fact the Fladen data (Steele, 1956) showed there could be great variability in production during this season.

To reproduce the effects of the breakdown, a high rate of mixing was applied to the conditions typical of a summer with a low amount of mixing. This gives the production rate shown by the lower line in Figure 4. The increase in production is very slow and it is likely that before the forty days had elapsed the thermocline would have broken down completely and production be ended. If, however, after a ten-day-period of intense mixing the rate becomes low again

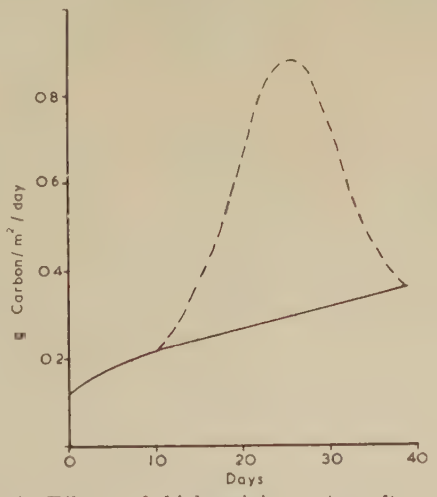

Figure 4. Effects of high mixing rates after summer conditions.

then the model shows rapid growth to give the large production indicated by the upper line in Figure 4. This implies that a genuine outburst occurs only when the period of intense mixing is sufficiently short to retain stratification and so allow production in the following calm period. This sequence of mixing occurred just once during the years when data are available for Fladen and it was in this year (1951) that high autumn production was found from the phosphate results.

Production estimates for the northern North Sea

The model can also be used to study the variations in those factors, such as sinking or photosynthetic rate, which have been kept constant in the previous discussion but its main application is to provide a hypothetical picture of the variations in production in the northern North Sea caused by the variations in vertical mixing. It can be shown that for this area the bottom temperature in summer is a good index of the general intensity of vertical mixing and on

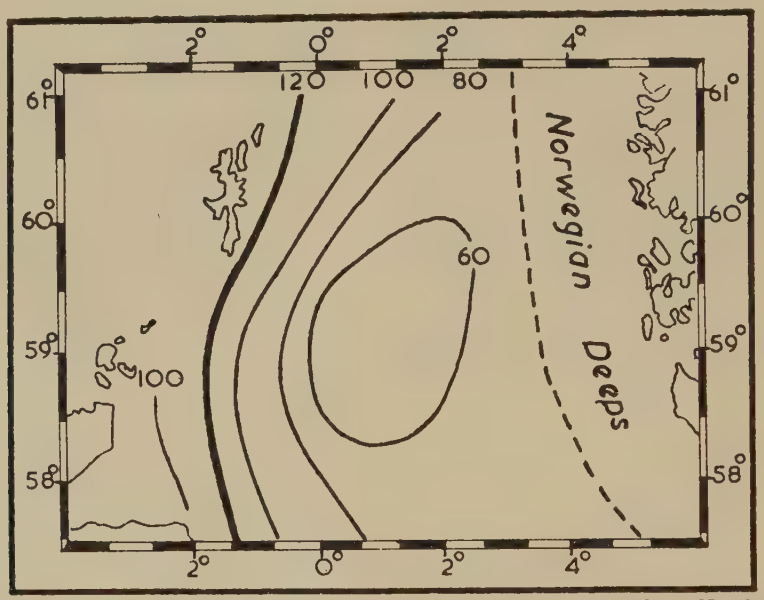

Figure 5. Expected yearly production of organic carbon in the northern North Sea.

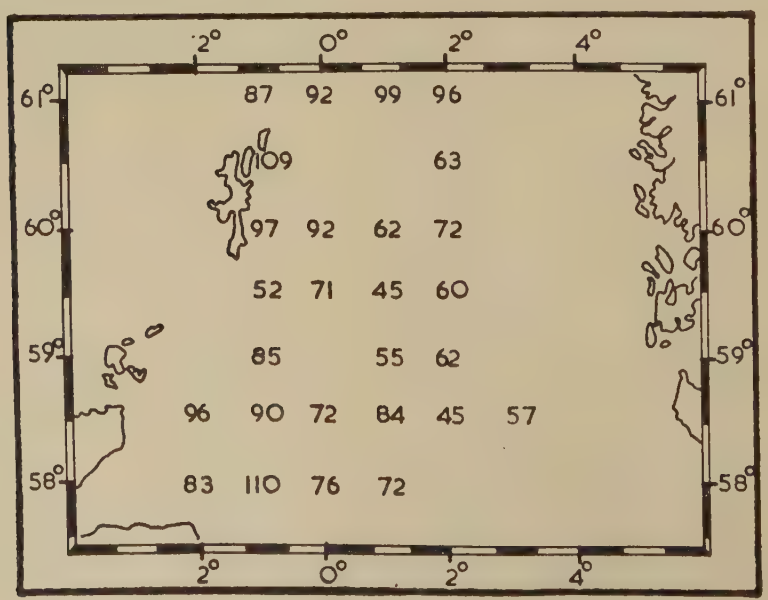

Figure 6. Mean yearly production estimates from phosphate data.

this basis, with a background of hydrographic theory, the chart of production shown in Figure 5 was constructed. The region over the Norwegian Deeps is excluded because of the considerable lateral water movement in that area.

The phosphate data collected over three years, 1954—56, provide a chart of estimates for the mean yearly production. The actual values are shown in Figure 6 and, although this is considerable variation (the figure of 52g. on the west side seems aberrant), the range of values and their distribution show a fair measure of general agreement. Further there is also good agreement for the different seasons; in both cases the higher yearly values depend on the higher summer production in the more coastal waters.

When there are disagreements the theoretical background of the model combined with the hypotheses about the hydrography can help to provide an explanation. Thus the phosphate data give occasional very high values for the spring outburst in the north-west corner of the area; values that are much higher than is possible according to the model. However, the general outline of the hydrography suggested that the model might break down in this area because of the intrusion of "oceanic" and therefore non-typical water; for these particular occasions the hydrographic data provided confirmation of this.

It is in this way that the model and the phosphate data together provide, both values for production, and an insight into the underlying mechanism.

Discussion

Three methods of estimating production have been mentioned, ^{14}C, phosphate, and mathematical model; none of these methods is adequate in itself and none is free from difficulties. It is perhaps the main conclusion from these North Sea studies that a combination of all three is necessary to give any degree of insight into the complex of physical factors controlling production. The ^{14}C technique must be used for detailed checks on the broader but more dubious interpretations from phosphate data. These in turn need a theoretical background and a study of the hydrography to provide reasons for the observed values and possible explanations of anomalous ones. In this way the three methods are complementary.

There is, however, one disadvantage common to these methods for measuring production quantitatively—none of them considers the particular species of plants and animals involved. The development of this subject has tended away from the traditional biological units and deals instead with such abstractions as "carbon content". This is due partly to the need to obtain quantitative results and also to achieve a generality which allows comparisons of results from different areas and at different times. It has produced, however, an apparent irrelevance to the results in other fields of plankton research. In particular it seems impossible to discover any link between quantitative studies of production and the data from the rapidly developing study of the effects of "metabolites", since this subject has gone to the other extreme in its laboratory experiments on single and often atypical species.

Beyond this, the abstract nature of the units used in these production studies makes it difficult to imagine the detailed steps to the higher tropical levels where the study of the species involved can no longer be neglected (even though a general outline is available, Harvey, 1950). To this extent these methods may lead to a dead-end. This difficulty is particularly serious when the attempt is made to relate the study of production to the problems of the fisheries; it is often possible to find apparent correlations but such comparisons are of very dubious value. For example, during the year in the northern North Sea the regions of maximum production are successively:—

(1) the Norwegian Deeps in March—April;
(2) the remaining area during the spring outburst in May;
(3) the western coastal waters during the summer;
(4) the deeper offshore waters in late autumn.

This cycle corresponds to the generally accepted migrations of autumn spawned herring in the area ("Banks" stock) but this movement can also be related to the current system and probably to a host of other factors. The same problems also occur in the southern North Sea (Cushing, 1955).

Again, the chart of yearly production shows fair agreement with the variations in growth-rates of haddock (provided by my colleague R. Jones), the smallest rates corresponding to areas of lowest production. But this pattern is also found in the bottom temperatures, and so the growth-rates may be linked either with feeding or with metabolic processes—or, once more, with some other unknown factor. It does not seem possible at present to think of any means by which these questions can be answered and this is probably the main problem facing the further development of the methods described here.

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C. 5.

The Primary Production in the Norwegian Sea in June 1954, Measured by an Adapted ^{14}C Technique

By Grim Berge

Introduction

The migrations of fish shoals in search of food is presumed to be influenced by the food supply in the different water bodies. When investigating the movements of the fish it might be of interest to know the extension and intensity of the productive and unproductive areas of the ocean, and as regards the resources of the sea knowledge of the primary production might also give valuable advice.

During the hydrographical cruise in the Norwegian Sea (21. May—1. July) with the research vessel "G. O. Sars" the opportunity was taken to carry out observations on the primary production.

From such observations at the hydrographical stations it would be desirable if charts of the productive areas might be drawn and the results brought in relation to the hydrographical, zooplanktonic, and fish investigations. There are, however, certain restrictions in regard to the method of observation:—

1. The sampling should cause a minimum of delay to the rather strained programme of the research vessel.
2. The method should give comparable values, where differences in production owing to changes in the condition of the weather were eliminated.
3. The results should be given for a mean day of the area investigated.

Furthermore, the method should be convenient to use in all weather conditions during day and night. To satisfy these restrictions a modification of Steemann Nielsen's (1952) ^{14}C method was used.

Methods

The primary production by algae is proportional to the light energy up to certain values characteristic for the time and area in question. Above this value the production increase per additional light unit is retarded. Jenkin (1937) estimated the proportionality to be valid up to 14,000 lux in the English Channel. Steemann Nielsen (1937) found maximum photosynthesis at 7000 lux for Danish autumn plankton.

In the waters near Bergen proportionality was found to be valid close upon 10,000 lux in plankton at 10 m. depth in May.

Within the proportional part of the light/production curve the production P is:—

$$P_T = K \int_0^T I_e \, dt \qquad (1)$$

where $\int_0^T I_e \, dt$ is the effective part of the incoming light energy during the time T (Sverdrup, 1953). K is a factor which is characteristic for the plankton population in question. If the light energy is measured in lux hours, then K means the production per lux hour. In this paper the production per litre and lux hour is referred to as the production capacity of the water.

When for the purpose of comparisons of the productions we let the identical mean day be represented by the arithmetic mean of n measurements of the effective incoming light energy, we have

$$\overline{S} = \frac{S_1 + S_2 \cdots + S_n}{n} \qquad (2)$$

Figure 1. The production capacity (10^{-7} mg./C/l./lux hr.) for three different depths. 24/V–25/VI, 1954.

where the S's for the sake of convenience represent the integrals of the effective light energy during a day.

When the $\overline{S}$ is known the production on the mean day $\overline{P}_d$ can be calculated from observations on the production capacity, by the formula

$$\overline{P}_d = K \times \overline{S} \qquad (3)$$

Direct measurements of the different S's would be difficult since very little is known of the effect on production of the qualities of light. Since, we have

$$S_1 = \frac{P_{d,1}}{K_1}, S_2 = \frac{P_{d,2}}{K_2}, \text{ and so on,} \qquad (4)$$

we get from (2) and (4)

$$\overline{S} = \frac{\frac{P_{d,1}}{K_1} + \frac{P_{d,2}}{K_2} + \cdots \frac{P_{d,n}}{K_n}}{n}$$

$\overline{S}$ may thus be calculated from n observations on P and K on different days and places within the area in question.

The formula is valid for the $\frac{P}{K}$ relation in depths corresponding to a certain percentage of light energy income.

In the Norwegian Sea nine such simultaneous observations on production and production capacity were performed with the purpose of estimating $\overline{S}$ in depths corresponding to 100 %, 26·5 %, and 7 % of the daylight penetrating to these depths.

The maximum light intensities on these days varied between 4,800 and 7,000 lux just below the surface, with a mean maximum of 5,800 lux.

Determinations of *P*

The penetration of the light into the sea was found by photoelectric measurements, using JERLOV's submarine photometer. The samples were taken with the Steemann Nielsen all glass water-bottle (AABYE JENSEN & STEEMANN NIELSEN, 1953) from depths corresponding to 100 %, 26·5 %, and 7 % of light penetration at noon. After the addition of accurately measured amounts of $Na_2{}^{14}CO_3$, the 100 ml. samples were placed in a thermostatic bath at the top deck of the ship. The samples were covered with neutral light filters corresponding to the depths where they were sampled. The exposition lasted from noon to midnight, subsequently the samples were filtered, dried, and stored for counting ashore. The results are given as $^1/_2$ day production in grammes carbon per litre.

Determinations of *K*

Determinations of the production capacity of the different water bodies were carried out at nearly 60 stations. Except for the simultaneous determinations of P and K the depths for sampling for these

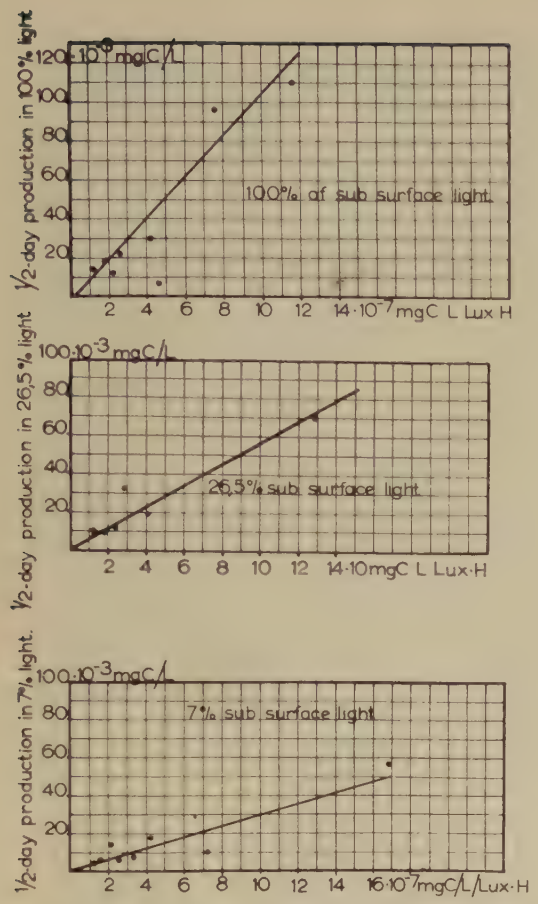
½-day production in 100% light
100·10⁻⁶ mgC/L
100% of sub surface light
2 4 6 8 10 12 14·10⁻⁷ mgC L Lux H
½-day production in 26,5% light
100·10⁻³ mgC/L
26,5% sub surface light
2 4 6 8 10 12 14·10 mgC L Lux·H
½-day production in 7% light
100·10⁻³ mgC/L
7% sub surface light
2 4 6 8 10 12 14 16·10⁻⁷ mgC/L/Lux·H

Figure 2. The correlation between 1/2 day production and the production capacity for three different light depths.

measurements were standardized at 0, 10, and 20 m. After addition of the $Na_2{}^{14}CO_3$ the samples were placed in a thermostatic bath under the illumination of artificial daylight of 4,800 lux. At the same level as the samples was a photoelectric cell and the light income during the 4 hours of experiments was recorded on an integrating lux-meter. After filtration and treatment as above, the final results were given as mg. carbon per litre and lux hour. Collodion filters and press-air filter apparatus were used for the filtrations (Calvin, *et al.*, 1949).

The light depths in question were in many cases fairly near to the depths of standard sampling for the production capacity determinations. In case of deviations K was determined by interpolations between the three measurements. From these K values and from the mean value of the corresponding P/K relation the production per litre and day were calculated as described above. Determinations of the production below 1 m². of surface are made by integrations of the production curves, between the depths for 1 % of daylight penetration and the surface.

Discussion of the method

As the observations of P in all experiments were carried out at very low light intensities, the estimation of $\overline{S}$ ought to be correct. According to Mosby (1932) the energy income on a clear day may be more than twice the value of that of a cloudy day. If the measurement of 4,800 lux at noon represents a dark day, light intensities might be expected to reach values of nearly 12,000 lux. In this case the light intensity for the surface sample will exceed the value until proportionality between production and light income exists. Estimations of $\overline{S}$ may, however, be made by means of neutral filters for light absorption, and by multiplying the S thus obtained by the filter factor.

If the production at all stations should be calculated on such an extreme day, the calculated P value at 0 m. would be a little too high. In the calculation of the production below 1 m²., namely, the integral of the production curve, the deviation from the correct value would hardly be detectable within the accuracy of the analyses. The total production below 1 m². on a quite clear day with subsurface illumination at noon of 12,000 lux, should therefore be approximately twice the value found for the mean day. During the cruise such extreme conditions hardly existed in the Norwegian Sea. The calculated differences, however, show the importance of making comparisons on identical days. According to Steemann Nielsen (1952) the estimation of P should be carried out by hanging bottles containing samples back into the sea. The use of daylight baths and neutral filters did not, however, show any significant differences from parallel samples in the sea for the filters used, as will be seen from the following experiment:—

26·5 % Daylight		7 % Daylight	
in the sea	in the bath	in the sea	in the bath
495 c/m	470 c/m	206 c/m	206 c/m
267 ,,	298 ,,	27 ,,	32 ,,
40 ,,	37 ,,	15 ,,	15 ,,

The differences are all within the accuracy obtainable by the ^{14}C technique in nature.

The calculated mean value of the incoming light energy will be applicable only within certain latitudes and seasons of the year. Within which latitudes and seasons the same $\overline{S}$ is to be used, depends on the accuracy claimed of the observations. The technique used has the advantage of making possible comparisons on any mean day. Differences in production owing to varying weather conditions is consequently eliminated. Observations may be performed at any time of the day and the delay at each station will be reduced.

Results

The results from the measurements of the production capacities are presented in Figure 1 for the three sampling depths of 0, 10, and 20 m. The charts have been made by drawing lines through points with the same production capacity. On basis of these charts the vertical distribution of production capacity has been drawn, as shown in Figure 3.

The most productive area is situated in the middle of the ocean and it can be traced as a river through the Norwegian Sea up to Spitsbergen. The north-western and south-eastern parts of the area investi-

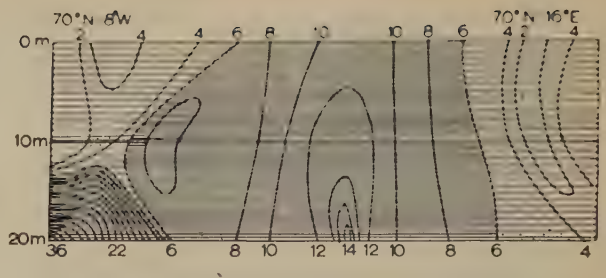

Figure 3. Vertical distribution of the production capacity along 70° N. All values are 10^{-7} mg.C/l./lux hr.

Figure 4. The production in g.C/day below 1 m². of the surface.

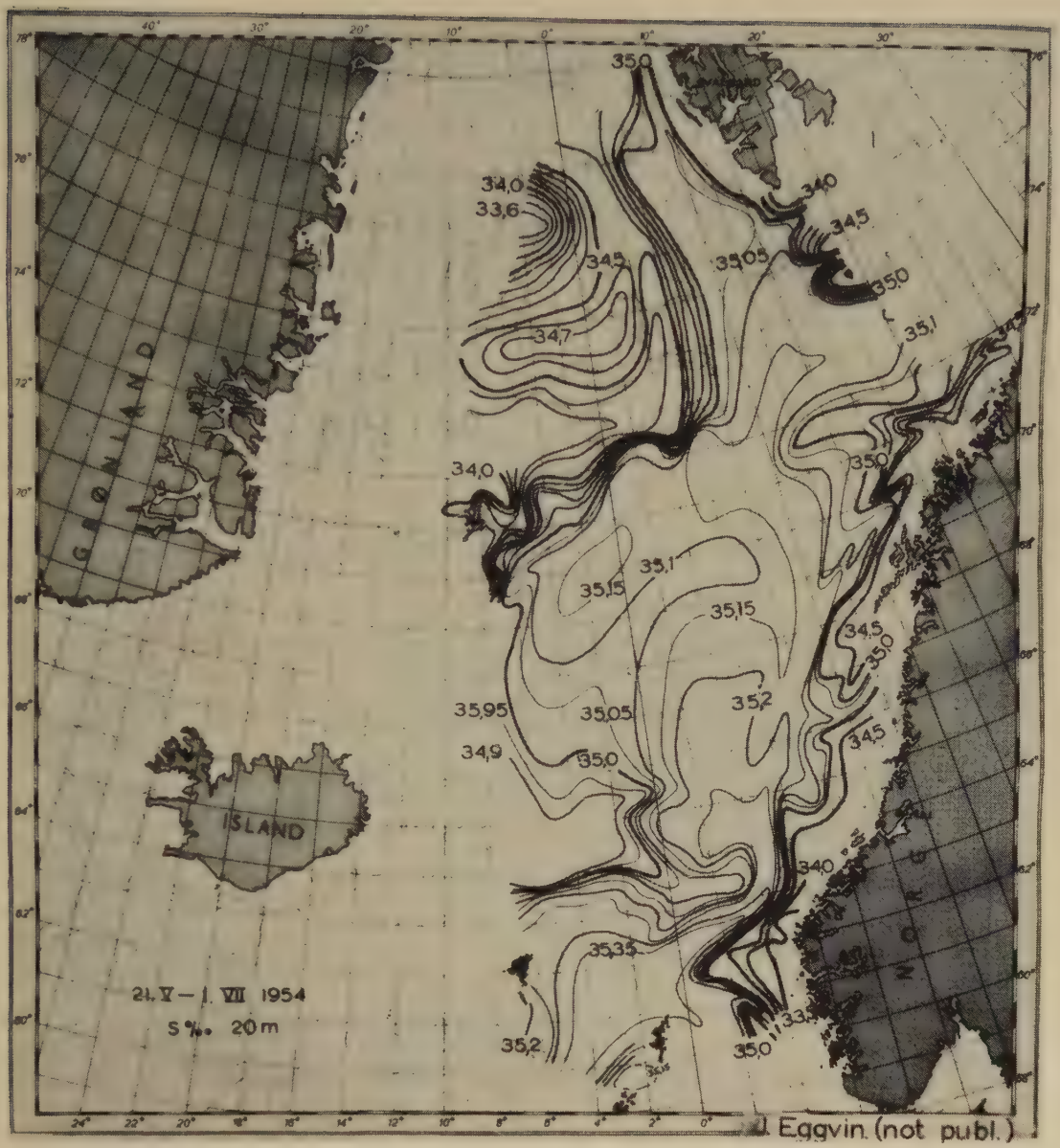

Figure 5. The salinity in 20 m. depth 21/V–1/VII, 1954. (With the courtesy of J. Eggvin.)

gated have a lower production capacity. The south-north distribution may, however, be somewhat influenced by the time factor, since the observations in the far north were carried out almost a month later than the southernmost observation.

In Figure 2 the results from the simultaneous $\frac{P}{K}$ relation are demonstrated graphically for the three depths in question. The mean value $\frac{P}{K} = \overline{S}$ is the line drawn. By means of these relations and the corresponding production capacities the production is estimated as described above. The production below 1 m². of surface is shown in Figure 4. The chart shows the same main features of productivity as shown in Figure 1, for the production capacities, but less pronounced. This is to be expected since a factor causing a rise in the production will be counteracted by the reduced transparency of the water in the total production below 1 m². Hence variations in the productivity will be most pronounced in Figure 1.

On the cruise, observations were taken of temperature, salinity, oxygen, and phosphate as well as samples of zooplankton and phytoplankton. This

Figure 6. The feeding area of the Atlantic-Scandinavian herring. (After J. J. Marty, 1956.)

material has neither been published, nor worked up yet, but Figure 5 is presented by courtesy of Dr. Eggvin. Atlantic water covering the central part of the Norwegian Sea is limited by the 35 $^0/_{00}$ isohaline. Arctic water is encountered in the west and northwest, and near the Norwegian coast the 35 $^0/_{00}$ isohaline marks the limit of the coastal water.

When comparing the production capacity for 20 m. depth (see Fig. 1c) to the hydrographical conditions (Fig. 5), it will be seen that the main production area is situated in the western part of the Atlantic water, with salinities below 35·15 $^0/_{00}$. The Atlantic water to the east is less productive. Production capacities above 6×10^{-7} mg.C/l./lux hour are shown hatched. The hatched area corresponds almost exactly with the distribution of Atlantic water with salinities between 35·00 and 35·15 $^0/_{00}$. The admixture of arctic water east of Iceland causes the decrease in salinity in the western part of the Atlantic water. The combination of these two water bodies, poor in production, seems to cause the very high-productive water in the central part of the Norwegian Sea. The same correlation with hydrography may be seen in the chart of total production, but here the facts are less pronounced.

A chart after J. J. Marty (1956) of the herring feeding area is shown in Figure 6. This chart being based on observations from more than one year an

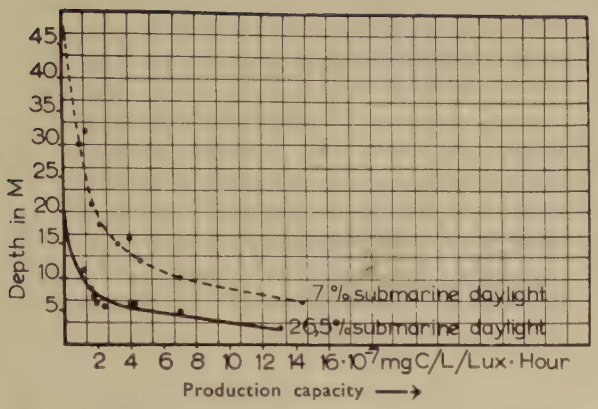

Figure 7. The correlation between depths of 26·5 % and 7 % submarine daylight and the mean value of production capacity for the water column above.

exact correlation to the production charts cannot be expected. The distribution of herring during the feeding period fits very well, however, with the productive area of the Atlantic water, and the eastern border of the feeding area corresponds closely with the isoline for the production of 0·8 g. carbon/day in Figure 4.

Since the measurements of the production capacity have been carried out under very identical conditions, some positive relation might be expected between the production capacity and the standing crop of phytoplankton and thus also a certain relation between the transparency of the water and the production capacity. This relation, however, only holds good in periods before the culmination of the blooming. In Figure 7 the production capacity is plotted against depths with 26·5 % and 7 % submarine daylight. There seems to be an almost logarithmic correlation between the depth of the light and the value of the production capacity in the water column above. It should therefore be possible by observations of the light to estimate the production capacity and the production for each of these depths.

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C. 6.

A Survey of Recent Danish Measurements of the Organic Productivity in the Sea

By E. Steemann Nielsen

Intensive investigations on organic productivity have been carried out during the last five years by the Danish Fishery Investigations. In Danish waters the *in situ* method (carbon-14 technique) has been employed exclusively. In northern waters the tank method and the simulated *in situ* method has been used during the summer cruises of the "Dana". In addition, the *in situ* method has been employed at three localities in the coastal waters near Greenland at all seasons of the year. A short survey of all these investigations will be presented here. Detailed evaluation of the material has not been completed at present.

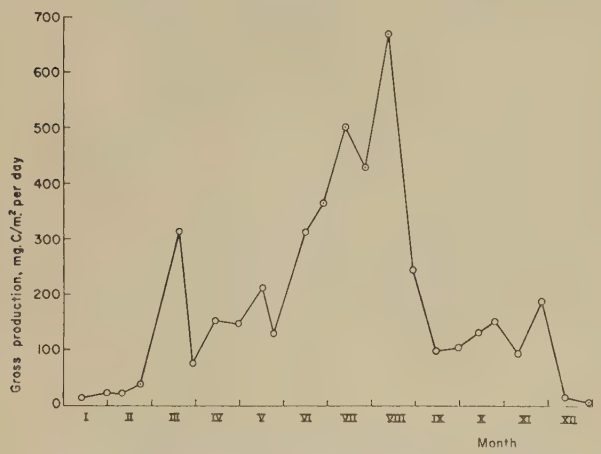

Figure 1. Gross production in the Great Belt — Halsskov Rev lightvessel — 1955.

Danish waters

Investigations have been carried out mainly in the Kattegat, the Great Belt, and some fjords, first of all in the Limfjord and in the Dybsø Fjord.

The measurements in the Great Belt were started in December 1952 and have been continued since that time. The measurements have been made at the lightship "Halsskov Rev" twice every month. The captains carried out all the field work. Water samples were collected in depths of 0, 6, 12, and 18 m. and, after addition of ^{14}C, were suspended in the same depths from noon to sunset.

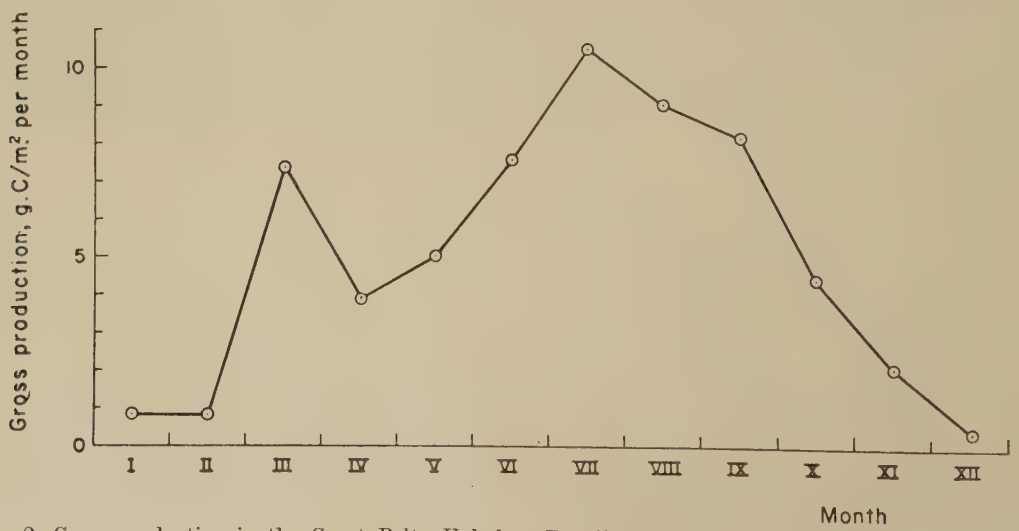

Figure 2. Gross production in the Great Belt—Halsskov Rev lightvessel. Monthly mean values 1953—1956.

Figure 1 presents as an example the annual cycle for the year 1955. A short spring maximum was found in March followed by a spring minimum. With the increasing temperatures during summer the rate of production increased, in all probability due to the increasing regeneration rate of the nutrient salts with temperature. The maximum rate of production was found in August. During autumn the rate of production decreased. It was possible to measure the rate of photosynthesis even during midwinter. Since the water layers are always very effectively stabilized, a standing crop of phytoplankton is able to survive near the surface.

Figure 2 presents a similar curve showing the average monthly rates of organic production for the years 1953—1956. The annual gross production did not vary much during these four years as shown below.

	Gross production g.C/m.² per year
1953.....	57
1954.....	60
1955.....	66
1956.....	56

The summer temperature seems to be the prime factor determining the size of the organic production, in all probability because the regeneration of the nutrient salts is dependent on temperature.

Figure 3 shows the total organic production during June-September for the four years as a function of the average surface temperature during these months. Variations in salinity, on the other hand, seem to be of minor importance only. It was impossible to find any correlation between salinity and rate of production.

According to Figure 2 the average monthly rate for September is high. However, in the years like 1955, showing a very high rate of production during the main part of summer, the rate during September was comparatively low—cf. Figure 1.

The table below presents the Danish localities from which annual series of productivity measurements are

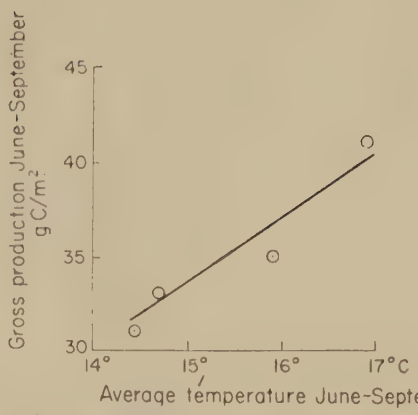

Figure 3. Total rate of gross production in the Great Belt during June-September as a function of the surface temperature.

now available. The two localities—the Sound and the inner part of the Isefjord—have been investigated previously with the oxygen technique.

	Gross production g. C/m.² per year	
Great Belt (Halsskov Rev)	60	(56—66)
Kattegat (Anholt Nord).....	61	(51—77)
Sound (near Elsinore).....	39	
Limfjord (Venø Bay)	105	
Isefjord (inner Broad).....	175	
Dybsø Fjord	6	

The lower production in the Sound as compared with the Kattegat and the Great Belt is in accordance with the magnitude of the standing crop of phytoplankton. The very high production in the Isefjord and the extremely low production in the Dybsø Fjord agree also with the standing crop. The last mentioned locality is extremely productive with respect to sessile plants. The very low rate of plankton production is not primarily due to the rather shallow depth. It seems to be due primarily to an antagonistic effect attributable to the sessile plants (*Chara*).

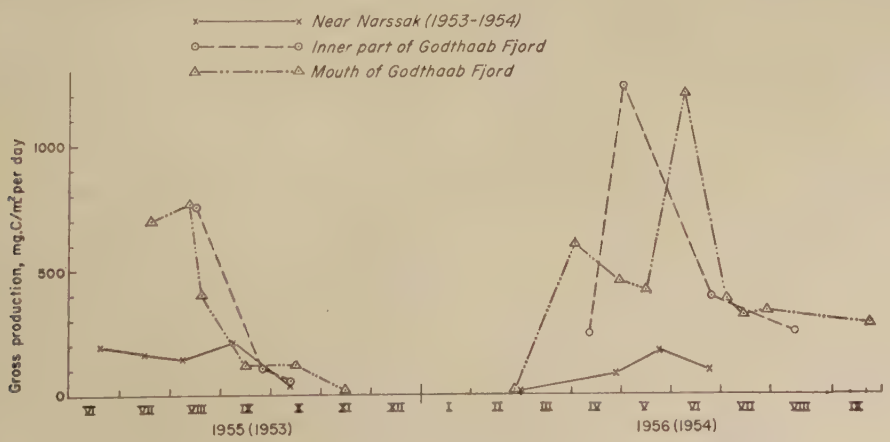

Figure 4. Gross production at three coastal localities, Greenland.

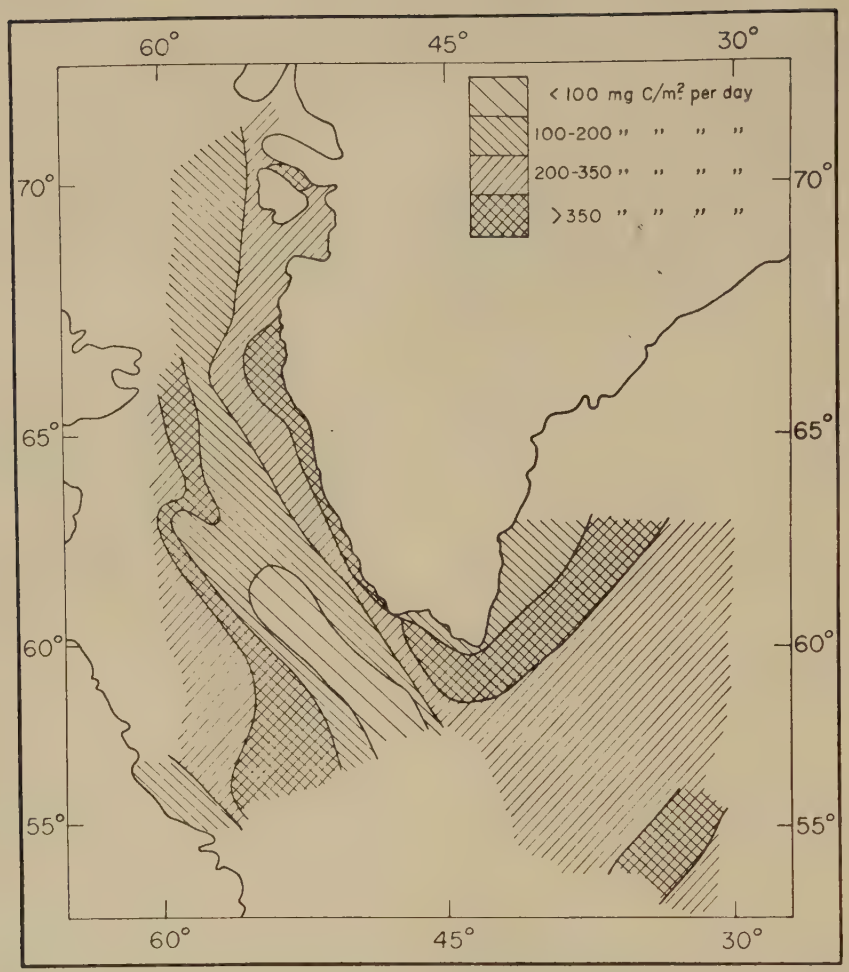

Figure 5. Gross production in the waters around Greenland July—August 1954.

Greenland coastal waters

Figure 4 presents the annual curves from the three coastal localities from Greenland: 1) outside Narssak, 2) mouth of the Godthaab Fjord, 3) the inner part of the Godthaab Fjord near Pisigsarfik. The table below shows the annual rates of production at the localities.

	Gross production g. C/m². per year
Outside Narssak	29
Mouth of the Godthaab Fjord ...	98
Inner part of the Godthaab Fjord	95

Narssak is situated in the southernmost part of West Greenland. The cold and light surface water of the East Greenland Current found outside the coast (cf. the chart, Figure 5) prevents any ascent to the surface of heavy and nutrient-rich oceanic deeper water. Such an ascent, on the other hand, takes place further to the north over the banks outside the Godthaab Fjord. Due to the considerable velocity of the currents produced by the tide, the whole of the Godthaab Fjord is influenced by the nutrient-rich water. The high rate of the production as compared with that near Narssak is easily explained.

The field work at the three coastal stations was carried out by Dr. E. Smidt and Mr. A. Horsted.

The waters round Greenland during the height of summer

Figure 5 presents the results of the "Dana" during July—August 1954. Similar investigations—although on a somewhat smaller scale—were made at the same time of the year in 1955, 1956, and 1957. The survey chart from 1954 is based on 63 stations in all. The results from the other years agree in principle fully with those from 1954. The relative distribution of the different water masses may, however, vary to some extent from year to year. July—August is in all

probability the best time of the year to compare the productivity of the different areas, if measurements can only be made once a year.

A high rate of production is met with at all the localities where fronts between two current systems are found. It is due to the formation of eddies bringing up nutrient-rich water to the surface. Such productive areas are thus found in the areas 1) at the front between the Atlantic Current and the water from the Irminger Sea, 2) outside the East Greenland Current, 3) at the front between the Labrador Current and the oceanic water in the centre of the Davis Strait. Very productive water is also found outside the coast of West Greenland between latitudes about 62° N. and 67° N. Over the banks here deeper water ascends toward the surface, thus enriching the photic layer with nutrients.

The oceanic water in the centre of the Davis Strait is rather unproductive during the height of summer. Earlier in the year the rate of organic production has very likely been considerably higher in this area. The water of the East Greenland Current is also rather unproductive at the height of summer. Due to the low salinity of the water in the photic layer no intermixing of importance with deeper nutrient-rich water is possible. After the end of the spring maximum, the rate of production therefore continues to be low for the rest of the year. Similar conditions are very likely to be found near the coast of Labrador. However, only one station was worked there by the "Dana" in 1954, but it may be noted that these conditions also prevailed in 1955.

During the cruise of 1954 the productivity work was carried out by Mr. V. Hansen.

C. 7.

Some Observations on Organic Production in the North-East Atlantic

By R. I. Currie

Introduction

In the course of some trials of new methods for studying the organic production in the sea, some preliminary results have been obtained, and although these do not yet form part of a major programme it is felt that they are worth noting at this stage.

The primary intention of this work was to make measurements in the open ocean and upon the natural populations therein, and the factors which have been measured and which will be dealt with in this paper are:—

(a) the rate of photosynthesis of the phytoplankton,
(b) the standing crop of phytoplankton,
(c) the light energy available for photosynthesis.

The observations at sea were made in the north-east Atlantic on the cruises of the R.R.S. "Discovery II" in September 1955 (Stations 3290—3346) and in September 1956 (Stations 3470—3485). The positions of these stations are shown on the chart in Figure 1 on which the rectangular symbols represent stations at which full series of observations were made, including measurements of photosynthesis. The circles represent stations at which measurements of standing crop alone were made.

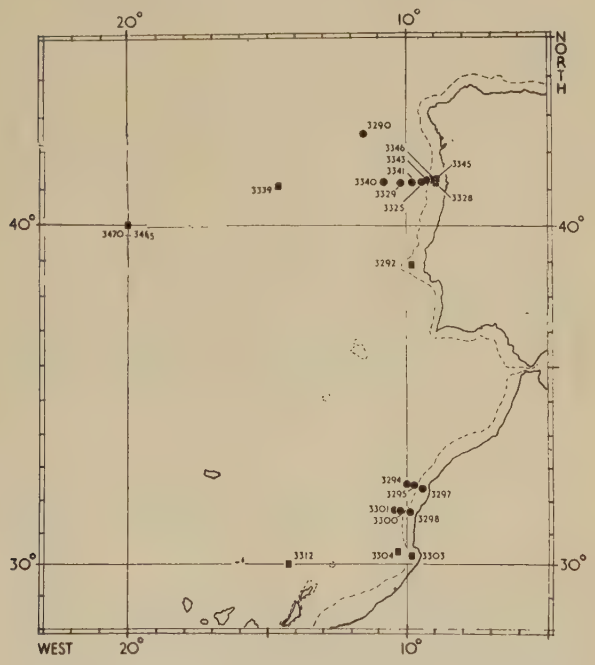

Figure 1. Chart showing the stations at which observations were made. Rectangles indicate ^{14}C experiments and pigment estimations. Circles represent pigment estimations only.

Methods

The measurement of carbon fixation by the phytoplankton

The rate of entry of carbon into the organic cycle through photosynthesis has been measured by the radioactive tracer technique which was introduced to this work by Steemann Nielsen (1952). The method consists in determining the uptake of $^{14}CO_2$ in a given period of photosynthesis. Essentially Steemann Nielsen's first method has been used but some practical modifications have been made to facilitate its use in routine work at sea. Other changes have been made only to ensure the efficiency of the method.

Although requiring skilful seamanship, it has been found possible to make observations in winds of up to force 7.

Samples are taken with a nylon lined (5 litre) water bottle. This is agitated to ensure that the sample is well mixed (a precaution found necessary to obtain good reproducibility between different exposure

bottles[1]). Two clear glass stoppered reagent bottles (175 ml.) are then filled with the sample and the tracer solution added. A small glass rod is also placed in each bottle to assist in the agitation of the sample. The bottles are then placed in clips and lowered to the depth from which they were taken, on a wire which is attached to some floats secured to a dhan buoy. The surface sample is supported in a cork float so that it is kept only slightly submerged. This whole arrangement is then cast adrift just before sunrise. A radar reflector on the dhan buoy enables the ship to keep in touch with it while other work is carried out. In this way it is possible to conduct the experiments with the least wastage of ship's time. At sunset, the buoy is picked up and the samples removed and filtered as quickly as possible.

The filters carrying the phytoplankton are then washed with 0·005 M. hydrochloric acid and dessicated overnight, and their radioactivity is measured the following day.

The results of the carbon assimilation measurements have been corrected in the manner proposed by Steemann Nielsen, allowing 6 % for the isotopic factor and 4 % for loss through respiration in the course of the experiment. The average dark assimilation varied from 1 % to 3 % in these experiments.

At the same time as the samples for the ^{14}C experiment are taken, a suitable quantity of the water sample is kept for an estimation of the standing crop of phytoplankton.

The measurement of the standing crop of phytoplankton

It must be emphasized that a measurement of pigment does not give a reliable estimate of the standing crop of phytoplankton in terms of the organic matter which it contains. However, the pigment in an alga is the agent responsible for the absorption of light energy, and so measurements of pigment can be used to give an estimate of the "photosynthetic potential" of the standing crop of phytoplankton. A measure of the "photosynthetic potential" is probably of more value for present purposes than a measurement of standing crop in terms of its organic matter content.

The method which we are using for this measurement is that of Richards and Thompson (1952). By measuring the extinction of a 90 % neutral acetone extract of the pigments, at five points in the spectrum (665, 645, 630, 510, and 480 mμ) the quantities of the pigments (chlorophylls *a*, *b*, and *c*, and two groups of carotenoid pigments) in the sample can be determined.

It has already been noted that water samples taken from the same sampling bottle as those used in the ^{14}C experiments, are used for the pigment measurements. From two to four litres of water are treated with magnesium carbonate, and then filtered through "Cella"[2]) filters of 0·5 μ pore size. Next the filters are placed in tubes and dessicated in vacuo in the dark. After a standard period of 24 hours dessication, the pigments are extracted for 24 hours in a solution of 90 % acetone (prepared by dilution of acetone fractionated over Na_2CO_3 and Na_2SO_3 and taken off at 56·5° C.). If necessary the extracts are centrifuged and then the extinctions are measured with a spectrophotometer.

The dessication of the samples in vacuo in the dark was found essential particularly if the samples had to be kept for more than 24 hours. Storage of the acetone extracts for longer than 24 hours is extremely unsatisfactory as rapid decomposition of the extract occurs, even in the dark.

Observations by these two methods were made in September and October 1955 and 1956. Altogether about 180 measurements of pigments have been made at 21 stations and experiments with the "carbon-14 method" have been conducted at 14 stations.

Observations on the Standing Crop of Phytoplankton

A wide variation has been found in the concentrations of the photosynthetic pigments in the different regions in which we have made observations. From minimal values found frequently at the oceanic stations, concentrations of up to 12·8 mg. of chlorophyll *a* per cubic metre have been found at stations in rich coastal waters, visibly discoloured by the density of phytoplankton. Even in these rich samples, however, chlorophyll *b* is found in very small quantities. Only on one occasion was a value as high as 1 mg./m³. recorded. In most cases the concentrations of chlorophyll *b* are barely detectable with the sensitivity of the method of estimation. Chlorophyll *c*, on the other hand, has been found to reach a concentration of 8 mspu/m³. (milli-specific pigment units, Richards and Thompson, 1952, p. 160) in richly populated coastal waters, and it was rarely found numerically lower than the concentration of chlorophyll *a* in oceanic water.

The principal "plant" carotenoids, the carotenes, fucoxanthin etc., were at one position off the Portuguese coast found to reach a concentration of 4·66 mspu/m³. but generally they occurred at much lower concentration <1 mspu/m³. in both oceanic and coastal waters. Estimations of the "astacin" type carotenoids have never exceeded 0·5 mspu/m³., and usually show insignificant concentrations.

It is not possible to say at present how much of the pigment is extracted from dead or dying organisms, and indeed below the euphotic zone there is evidence of still considerable quantities of pigment (such as has already been observed by Riley, Stommel, and Bumpus, 1949). However, for the present the assumption has been made that these measurements of

[1]) It is suspected that this may be due to a small-scale patchiness of the phytoplankton, a phenomenon which has been observed visually on other occasions.

[2]) These filters, which are insoluble in acetone, can be obtained from Membranfiltergesellschaft, Göttingen.

pigment do in fact represent the photosynthetic potential of the "growing crop" in the euphotic zone.

The ratio of clorophyll *a* to chlorophyll *c*

It has been noted that the two pigments found in any abundance in the present samples, were chlorophyll *a* and cholorophyll *c*. If we plot the ratio of chlorophyll *a* to chlorophyll *c*, it is at once evident that

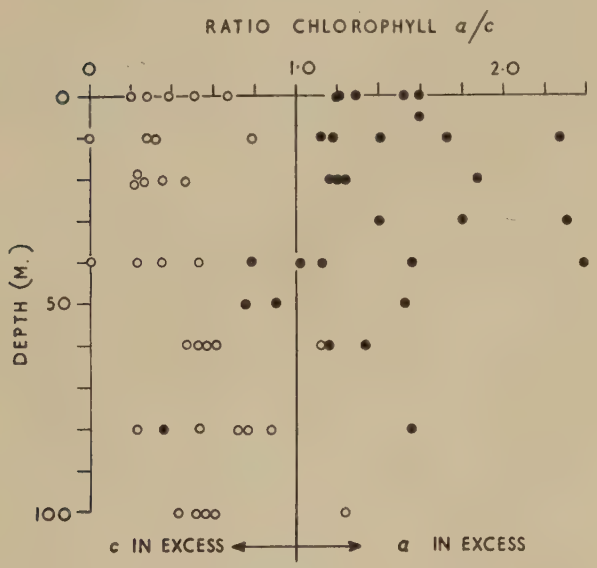

Figure 2. Ratio of chlorophyll *a* to chlorophyll *c* plotted against depth for five coastal stations (3303, 3304, 3328, 3345, and 3346) indicated by dots and five oceanic stations (Stations 3312, 3339, 3470, 3472, and 3481) indicated by open circles.

considerable variation occurs. The greatest contrast is between "coastal" and "oceanic" stations. At the former, there is a marked predominance of chlorophyll *a*, whereas at the latter chlorophyll *c* is frequently numerically in excess. The comparison between these two pigments is, of course, complicated by the fact that the units of measurement are not directly comparable. Chlorophyll *a* is measured in mg. per m³., whereas chlorophyll *c* is measured in mspu per m³. Although this may affect the absolute comparison it does not destroy the relative comparability. Figure 2 shows the ratio plotted against depth at 10 stations, five coastal and five oceanic. Of 59 offshore observations the mean ratio chlorophyll *a*/*c* was 0·79 whereas in 31 inshore observations the mean was 1·34. It appears, therefore, that at most of the stations well out in the open ocean, chlorophyll *c* is numerically the predominating pigment. The most probable explanation of the variation in this ratio, seems to be the difference in specific composition of the phytoplankton standing crop.

The pigments as a measure of photosythetic potential

Ryther (1956b) in using chlorophyll *a* alone as his measure of "photosynthetic potential" has conformed with the generally accepted theory that chlorophyll *a* is the pigment of prime importance in the photosynthesis of the algae. The light energy which it absorbs can be utilized directly in the photochemical reaction. There is still some doubt about the precise rôle of the other pigments. It has been established, e.g. by Tanada (1951) that the energy absorbed by fucoxanthin is utilized in photosynthesis, but Duysens (1951) and others show that such energy absorbed has to be passed through chlorophyll *a* before it can enter into the chemical part of the reaction. It is not, therefore, possible at present to define the precise importance of all the accessory pigments, but on the existing evidence for at least a partial utilization of these pigments (fucoxanthin) and also in view of the relatively large concentration of chlorophyll *c* found in oceanic waters it has been considered, for the present, more advisable to take the total of the photosynthetic pigments as a measure of the "photosynthetic potential". That this is a reasonable assumption is also borne out by results, for the agreement between carbon fixation and "photosynthetic potential" was not so good when chlorophyll *a* alone was used as the measure. The measure of "photosynthetic potential" which is used is, therefore, the sum of chlorophyll $a + b + c +$ the "plant" carotenoids (those referred to as "non-astacin type" by Richards and Thompson, 1952).

It should be noted that being a sum of chlorophyll *a* and *b* expressed in mg./m³. and chlorophyll *c* and the carotenoids expressed in mspu/m³., this total is a slightly unreal quantity.

Results of the Carbon Fixation Measurements

In September—October 1955 a series of successful measurements were made at varying distances from the coasts of Portugal (in 41° N.) and Morocco (in 30° N.). A wide variation was found in the total amount of carbon fixation below unit area of sea surface at these different places. Simultaneous measurements of the photosynthetic pigments showed a very similar variation (Table 1) and if the total pigment in the euphotic zone (taking this arbitrarily as the greatest depth of carbon assimilation), is calculated, then the ratio of the total carbon fixation to total photosynthetic pigment (A/B Table 1) is surprisingly constant.

At nearly all of the stations the carbon assimilation increased from the surface to a maximum at some depth, and then decreased below this reaching zero at depths varying from 30 to 120 metres. In some cases two subsurface maxima were found.

These features do not, however, take into account the distribution of the phytoplankton standing crop in the water column, and to get an estimate of the

Table 1. Carbon assimilation and pigment measurements at Stations 3292 to 3345 in September and October 1955

Station Number	Position	Lat.	Secchi disc m.	*A* Total C fixed mg./m.²/day	*B* Total photosynthetic pigment units in euphotic zone/m.²	*A/B*
3292 Sept.	Off Lisbon on shelf	39° N.	17	189	189	1·00
3303 Sept.	Agadir on shelf	30° N.	9	474	209	2·27
3304 Sept.	Agadir edge on shelf	30° N.	19	399	162	2·46
3312 Sept.	200 miles W. of Agadir	30° N.	48	107	84	1·27
3328 Sept.	Oporto 15 miles off	41° N.	8	1115	849	1·31
3339 Sept.	Oporto 200 miles off	41° N.	33	165	129	1·28
3345 Oct.	Oporto 15 miles off	41° N.	21	531	396	1·34

actual efficiency of photosynthesis at different depths, it is necessary to correct these figures to obtain the effect which would be obtained with a homogeneous population in the water column at the various stations. This has been done, by calculating the carbon fixation in terms of one unit of total photosynthetic pigment for each depth of sampling, and the results show some particularly interesting features.

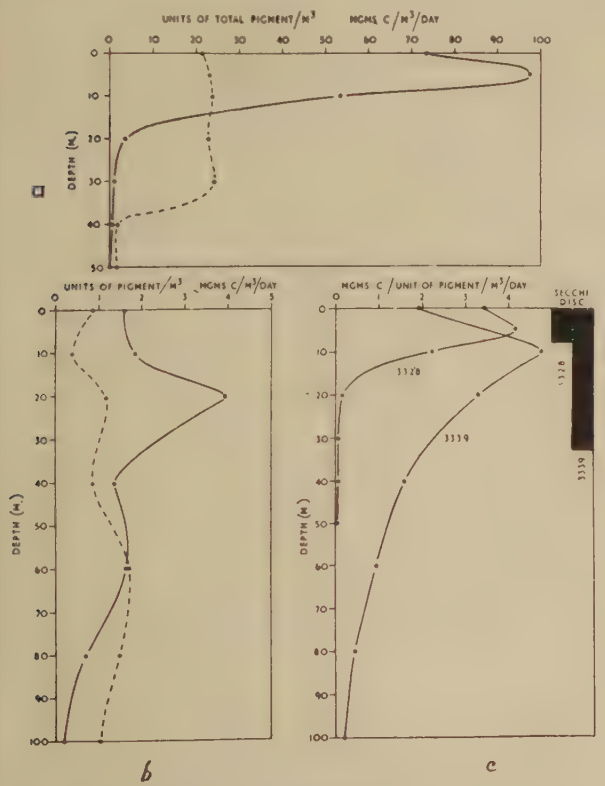

Figure 3. *a.* Total carbon assimilation (plain curve) and total photosynthetic pigment (broken line) at Station 3328, 15 miles off the Portuguese coast in 41° N. *b.* Total carbon assimilation and total photosynthetic pigment at Station 3339, 200 miles off the Portuguese coast in 41° N. *c.* Carbon fixation per unit of total photosynthetic pigment at Stations 3328 and 3339. The Secchi disc observations are also shown.

The plain curve in Figure 3a, shows the carbon fixation in mg. C/m³./day and the broken line, the total pigment at Station 3328, some 15 miles from the Portuguese coast in 41° N. Figure 3b shows the same for another station 3339, made in similar weather conditions 200 miles west of the coast in the same latitude. It should be noted that the scale of the abcissa of these two diagrams differs by a factor of ten. Clearly the carbon fixation at the oceanic stations was but a minute fraction of that at the coastal station and there was a similar difference in the standing crop.

In Figure 3c, is shown the calculated carbon fixation per unit of photosynthetic pigment for these two stations, both curves being on the same scale. Evidently from the remarkable similarity of these two curves the amount of photosynthesis in terms of unit pigment was of the same order of magnitude at the two stations although it extended to a much greater depth at the oceanic station. The two curves show plainly the inhibiting effect probably due to the stronger light at the surface, and beneath the subsurface maximum, a steady logarithmic decrease similar to that found in the cultures used by JENKIN, 1937.

A similar agreement was found between the stations in 30° N. at 240 miles, and 15 miles from the coast of Morocco.

The close relationship between these curves and JENKIN's energy flux calculations suggested that an agreement might be found under natural conditions between the available light energy and this measure of photosynthesis per unit of photosynthetic pigment.

To this end some further experiments were conducted at the next opportunity, in September 1956. If a relationship were to be investigated between these two parameters it was obviously necessary to eliminate as many other variables as possible.

It was particularly necessary to avoid great or rapid fluctuations in the population or its environment while the measurements were being made, and to avoid such variations a position was chosen in 40° N., 20° W., where no strong currents were prevalent and where influence from coastal regions and island masses could be expected to be minimal.

It proved possible to make repeated observations at this position over a period of three weeks. The

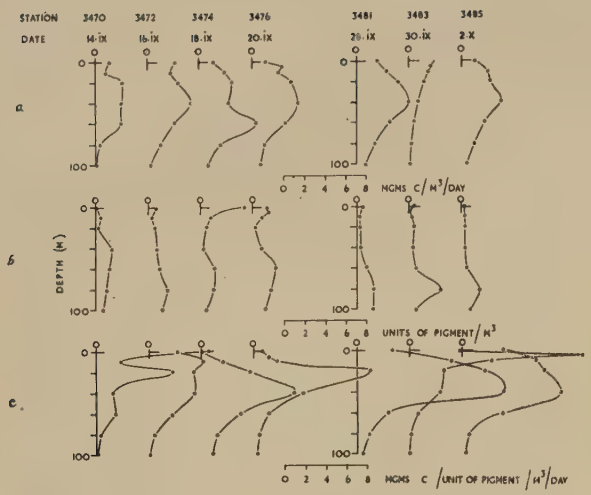

Figure 4. Repeated observations at position 40° N., 20° W. in 1956.
a. Total carbon assimilation.
b. Total photosynthetic pigment.
c. Carbon fixation per unit of photosynthetic pigment.

first four observations (Stations 3470, 3472, 3474, and 3476) were made with an interval of a day between each, and then they were broken off for a week after which three subsequent observations (Stations 3481, 3483, and 3485) were made as before, with a day interval between each.

The hydrological conditions remained fairly constant throughout the period of observation. During the first week a well marked thermocline lay between 30 and 70 metres, and above this a well mixed convection layer which warmed from 20·1° to 20·5° C. over the first four observations. A gale which occurred in the subsequent week deepened and intensified the thermocline so that in the second group of observations it lay between 50 and 80 metres with a somewhat steeper gradient. The convection layer was also lowered in temperature to between 19·8° and 20° C.

The standing crop of phytoplankton in terms of the total photosynthetic pigment varied between 90 and 190 units/m². (Table 2, Figure 4b), and the total carbon fixation (Table 2, Figure 4a) varied rather more from 320 to 86 mg.C/m².

In terms of unit pigment, the carbon assimilation curves (Figure 4c), although showing the same general features at each station, tend to be somewhat irregular, particularly near the surface. It is thought that this must be a result of slight inaccuracies in the pigment estimations which were only just above the limits of error of the method. Below the subsurface maximum they show a fairly uniform logarithmic decrease, but it will be observed that the subsurface maximum varies considerably in magnitude. So far the reason for this is not apparent, although when the detailed light measurements are available (see below) it may be possible to find the cause of this anomaly.

Table 2. Carbon assimilation and pigment measurements at position 40°N., 20°W. in September-October, 1956

Station	Date	*A* Total carbon fixed mg. C/m.²/day	*B* Total photosynthetic pigment units/m.²	*A/B*
3470.....	14.IX	162	116	1·40
3472.....	16.IX	252	175	1·44
3474.....	18.IX	297	127	2·34
3476.....	20.IX	286	188	1·52
3481.....	28.IX	320	152	2·11
3483.....	30·IX	86	101	0·85
3485.....	2.X	222	91	2·43

Light Energy

The method of measurement of light energy

To determine the amount of light energy available for photosynthesis a continous record of the incident sunlight was kept throughout the ^{14}C exposures in 1956, and when conditions permitted measurements were made also of its submarine penetration.

Since a reliable calibration of the photometers used has not yet been completed, a detailed account of the results of this part of the work is not yet available. Some of the results, however, are already clear enough for broad conclusions to be drawn from them. As these cannot be materially altered in consequence of the more accurate calibration of the light meters, it is considered justifiable to report on them here.

The continuous record of sunlight was made with a barrier layer selenium cell in a watertight case mounted on gymbals behind an opal glass diffusing screen. The set up was similar to that used by Jerlov, 1951. Recordings were made on a Cambridge recording potentiometer, the current being taken from the cell through a suitable potential divider.

The incident light diffused by the opal was filtered through a green Schott VG 9 filter, and periodical comparisons were made through a red (RG 1) and blue (BG 12) filter to determine the approximate spectral composition of the light on days with widely varying cloud conditions.

A rough calibration of the cell was made using Ångström's method (in Pettersson & Poole, 1937) on the only two suitable occasions which presented themselves.

Relationship to photosynthesis measurements

Of the three stations in the second period of the 1956 observations, stations 3481, 3483, and 3485, the first and last (3481, 85) were done on bright sunny days with some cloud. The total incident energy (400—700 mμ) was roughly about 180 g.cals./cm². on each of these days. The intermediate station, however, was made on a very dark overcast day, with intermittent rain and squalls throughout the day.

On this occasion the total incident energy was about 40 g.cals./cm². slightly less than a quarter of that on the two bright days.

The total carbon assimilation (Table 2) on the dark day was about $^1/_4$—$^1/_3$ of that on the two bright days, and yet other factors apart from light can be assumed to have been relatively constant. Further it will be noticed that on this dark day the carbon assimilation showed no subsurface peak (Figure 4a) and an examination of the curves for carbon assimilation per unit of total pigment (Figure 4c) shows that on the dark day the whole curve shifted bodily towards the surface, so that the maximum lay at 5 m. compared with 40 m. on the two bright days. This is virtually what might be expected on a theoretical consideration of the effects of reduced light intensity (e.g., see Steemann Nielsen, 1954, p. 317).

It is further of interest to note that even with this rough calibration of the photocells, the optimal intensity below which there is a straight line relationship between light energy and photosynthesis (found by Jenkin at 1·8 g.cals./cm²./hr.) would lie between 5 and 10 metres on the dark day and from 30 to 35 metres on the bright days—the approximate depths of the maximum carbon assimilation.

The Interrelationships of Standing Crop and the Rate of Photosynthesis

The results of these experiments show clearly the pronounced differences which exist between the eutrophic and oligotrophic environments of coastal and oceanic waters. The stations in coastal waters were characterized by a high rate of total carbon fixation and a large standing crop of phytoplankton, whereas at the stations in oceanic waters both the rate of carbon fixation and the standing crop were low. The estimated quantities of carbon fixation were of the same order of magnitude as those found by Steemann Nielsen (1952, 1954) in similar regions.

The greatest interest, however, arises from the calculated rate of carbon fixation per unit of photosynthetic pigment. In respect of this, the stations in the two types of environment showed a much greater similarity. The rate of photosynthesis per unit of pigment was of similar magnitude both in coastal and in oceanic waters, and since in the latter the euphotic zone is of greater vertical extent, the total rate of photosynthesis in terms of unit pigment and below unit area of sea surface was greater at the "oceanic" than at the "coastal" stations.

On the strength of what evidence we have that light energy may have been the factor of greatest importance in controlling the photosynthetic rate measured in these observations, it appears that a fairly close relationship may exist between standing crop, photosynthetic rate, and the light energy available for photosynthesis, independently of the environment.

We have not, however, taken temperature into account in this comparison. To what extent it must affect the photosynthetic rate it would be inadvisable to estimate, as one is dealing with such entirely different populations of algae in the two environments. An example of the magnitude of the difference, gives a rough idea of its probable importance. The temperature at the "coastal" station 3328, decreased uniformly from 15° C. at the surface, to 13° C. at 50 metres. At the oceanic station 3339 the temperature was constant at 19·5° C. down to 40 m., and then decreased regularly to 13° C. at 100 metres. Thus in the upper 60 metres of the water column at the oceanic station the temperature was a maximum of 6° C. higher than at the coastal station. The importance of temperature is likely to be greatest at the higher light intensities nearer the surface.

If the individual units of standing crop (as photosynthetic pigment) were photosynthesising at the same rate independently of their environment and the rate was controlled principally by the available amount of light, then the question is why the standing crop remained at a low level in these offshore waters. During the three weeks we were on station in September 1956, there was little significant change in the standing crop. We must look for an explanation of this discrepancy partly in the ^{14}C measurements, and partly in other factors which have a controlling influence on the standing crop. Firstly, it must be remembered that photosynthesis is not synonymous with growth, and that although carbohydrate may be synthesised, the absence of other essential factors may prevent its utilisation in the growth of the organism. One might expect that as the oceanic organisms are living at very low nutrient levels, their growth-rate would be arrested on this account, and that a large part of the carbon which is photosynthesised into organic matter may be lost again through respiratory and excretory mechanisms. With the organisms living in the coastal waters, however, the environment provides a plentiful supply of nutriment, and with an unrestricted growth-rate they may be able to make more use of the carbon which is photosynthesised and incorporate the bulk of it in new plant tissue.

Ryther (1954) concluded from some experiments on a culture of *Chlamydomonas* sp. that the rate of respiration varied with the state of nutrition of the culture. He notes that it was 5—10 % of photosynthesis during the exponential growth phase of the culture and that it increased to 50 % when the population asymptote was reached. It is questionable, however, if these results can be compared with what occurs in the sea, as the oceanic organisms may well be adapted to low nutrient conditions, and whether or not the post-exponential growth phase is reached we do not know.

Some experiments were tried during the observations in September 1955, to find out whether alteration of the nutrient content of the water would have any effect on the photosynthetic rate.

Water samples were taken in the normal manner for the ^{14}C experiments except that at each depth an additional pair of exposure bottles were filled, and

to these was added a solution of KH_2PO_4 and Na_2NO_3 of a strength sufficient to increase the concentration of phosphate in solution to 10 mg.at./m³. and of nitrate to 100 mg.at./m³. The exposures were then made normally, hanging two pairs of bottles at each depth, to compare the difference between the ^{14}C measurements in the raw sea water and in the enriched sea water. The period of exposure was from sunrise until sunset, and no significant increase of photosynthetic activity was observed in the enriched samples, although this period of exposure may hardly have been long enough to permit the enrichment to take effect. Furthermore, it appears from Doty's (1955) observations that these nutrient additions may have been excessive.

Some doubt still exists as to whether the ^{14}C method measures *gross* or *nett* photosynthesis, that is whether it measures the total photosynthesis or photosynthesis minus respiration. Steemann Nielsen and Al Kholy (1956) subscribe to the view that gross photosynthesis is measured whereas Ryther (1954) is of the opinion that nett photosynthesis is measured.

The present results can throw no further light on this question and until further investigations have been made it is not possible to say whether they represent gross or nett photosynthesis. If they do represent *nett* photosynthesis, however, the question of a respiratory rate which varies with the state of nutrition of the organisms can play no part in the explanation of the stable standing crop at the oceanic stations.

The standing crop is a momentary equilibrium between the processes of increase and decrease, and if it remains level then the process of increase must be balanced by an equivalent decrease. Besides respiration, organic matter can be lost from the standing crop by the processes of excretion, death of the organisms, sinking from the euphotic zone, and grazing by herbivorous animals. Generally excretion is considered to be at a minimum in the algae (Fogg, 1953) but if any of the other factors were markedly more important at the oceanic than at the coastal station, they may be contributory to the stability of the standing crop at the oceanic station. Of these processes, it appears likely that grazing by herbivorous animals may be a factor of great importance, but as yet we have too little knowledge of this process, both qualitatively and quantitatively in the oceanic and coastal environment to draw further conclusions.

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C. 8.

Données Récentes sur la Fertilité Elémentaire en Méditerranée

Par F. Bernard

Dans le domaine qui nous occupe, les recherches en Méditerranée sont en retard par rapport aux connaissances acquises depuis peu sur les océans tropicaux et les mers nordiques. Cependant, diverses études, poursuivies de 1936 à 1957, apportent des résultats plus étendus et plus quantitatifs que les investigations antérieures. C'est pour mettre les océanographes au courant de ces résultats que je publie la présente note. Elle serait sans grande utilité si la Méditerranée était très spéciale et par trop différente des autres mers. Mais beaucoup de ses propriétés: grande dominance des *Coccolithus* en volume, abondance des *Exuviella* et *Nostoc* à certains niveaux, etc. se retrouvent largement dans les parties chaudes des océans, et plusieurs des faits exposés ici ont donc une portée générale.

I. Bref Historique

Au siècle précédent, et jusqu'à Nathanson (1910) et même à Pavillard (1937), divers spécialistes du phytoplancton, habitués à le récolter au filet fin, s'étonnaient de l'extrême pauvreté de la Méditerranée estivale en diatomées et grands péridiniens. A n'en juger que par ces deux groupes, on se demandait où le zooplancton pouvait trouver quelque chose à manger de juin à octobre.

Pourtant, ces auteurs ignoraient, ou peut-être oubliaient, les travaux d'un précurseur de grand mérite, H. Lohmann. De 1903 à 1920, il montre la nécessité d'étudier l'eau de mer, dont les protistes dominants (en Sicile et dans l'Atlantique tropical) sont de minuscules flagellés de moins de 30 μ, échappant aux filets les plus fins. Mais Lohmann les récoltait, soit par centrifugation de l'eau, soit par filtration sur des branchies d'invertébrés. Ces procédés sont peu quantitatifs et font perdre de 30 à 80 % des cellules, surtout dans les petites espèces à coque gélatineuse.

E. Steemann Nielsen (1933) applique au plancton marin le procédé d'Utermöhl, inventé pour les micro-organismes d'eau douce (sédimentation de l'eau formolée au-dessus d'un microscope inversé). Cette méthode est la plus précise, et, dès 1936, Ercegovic l'emploie pour le phytoplancton adriatique au large de Split. En 1936 également, Hentschel, grâce à l'expédition du "Meteor", confirme les données de Lohmann sur l'importance des flagellés calcaires dans l'Atlantique chaud, mais en procédant encore par centrifugation.

C'est aussi en 1936 que le Professeur L. Fage m'a orienté vers le nannoplancton méditerranéen. Pour en mieux connaître la quantité moyenne annuelle, des dosages méthodiques furent effectués, de 6 à 12 milles des rivages, au large de Monaco et de Banyuls (1936—38) puis d'Alger (F. Bernard, 1946).

A bord de la "Galathea" (1952), E. Steemann Nielsen parcourt divers océans, surtout dans la zone tropicale, et résume (1952—54) les résultats obtenus sur la photosynthèse au large grâce au carbone radioactif ^{14}C. Ses conclusions, d'un exceptionnel intérêt, confirment les données sur la vitesse de multiplication des unicellulaires végétaux. Si, à l'ouest de l'Afrique ou en mer froide, le carbone organique peut doubler en 24 heures, dans l'ensemble des mers chaudes, il n'augmente que d'un tiers à un sixième par journée.

Pour la Méditerranée, les faits les plus récents proviennent de croisières de divers navires dans le sud de cette mer. Au large de l'Algérie, le "Président Théodore-Tissier", puis le "Professeur Lacaze-Duthiers" et deux escorteurs de la Marine française récoltent plus de 2.000 échantillons d'eau pour F. Bernard, qui en publie les analyses en 1951 et 1956 et explore le détroit de Gibraltar en 1954. Il participe enfin à la croisière de la "Calypso" (juillet 1955), allant de Marseille en Grèce par la Sicile et la Crète.

Les lignes suivantes rappellent d'abord nos données antérieures sur l'Algérie occidentale et Monaco et ajoutent des chiffres et remarques inédits sur la Méditerranée orientale, qui, par sa fertilité élémen-

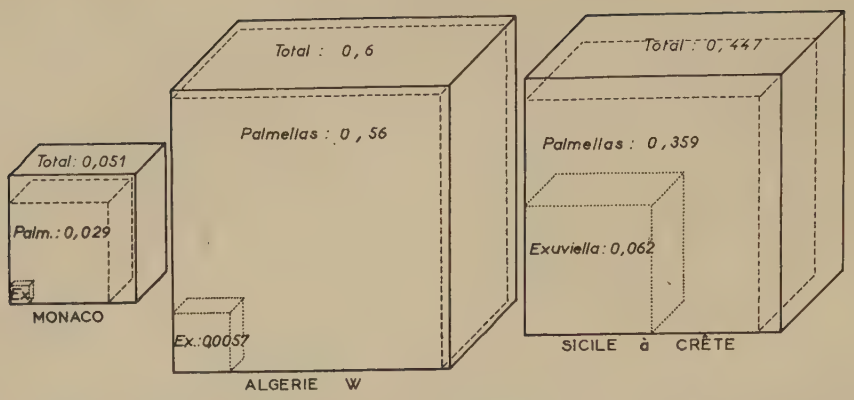

Figure 1. Schémas cubiques comparant les volumes moyens de phytoplancton en été entre Monaco, l'Algérie W. et la coupe Sicile-Crète (voir légende de la Figure 2). Le volume total est exprimé en millimètres-cubes par litre d'eau, pour la moyenne générale de 0 à 2.500 mètres de profondeur en chaque région. Ce volume est calculé d'après le nombre et la dimension moyenne des divers flagellés, plus les *Nostoc* (communs) et les diatomées (très rares sauf à Monaco).

On remarquera la très forte prépondérance en volume des palmellas de *Coccolithus fragilis* en Algérie (93,6 % du volume total). De la Sicile à la Crète, ce pourcentage, encore grand, tombe néanmoins à 80 % à cause de la concurrence des *Exuviella*, surtout nette de 250 à 800 mètres. En Algérie, les *Exuviella* sont dix fois moins nombreuses, et presque négligeable au large de Monaco.

taire entre la Sicile et la Crète, se révèle à peine moins productive que les parages de l'Algérie.

J. Brouardel a commencé depuis peu à Monaco l'application du procédé Steemann Nielsen au carbone-14. Ces recherches ne sont encore publiées qu'en partie, nous en tiendrons compte dans nos conclusions (voir la fin du paragraphe IV).

II. Organismes Dominants du Plancton Unicellulaire

Les groupes importants, jouant un rôle notable dans la photosynthèse et la fertilité totales, vont être énumérés par ordre d'abondance décroissante, d'après leurs moyennes de 0 à 2.500 mètres:—

1. Coccolithophoridae

De loin les plus abondants et pérennes, à toute profondeur. Comme densité, leurs cellules ne font que 16 à 50 % du total, mais, en raison du volume de l'espèce dominante, *Coccolithus fragilis* Lohm. (2.000 à 4.000 μ^3 par élément), les flagellés calcaires assurent partout, en Méditerranée, de 80 à 99 % du volume du phytoplancton par litre. Voici les moyennes annuelles obtenues de 0 à 2.500 mètres:—

Monaco: 92 % de Coccolithophorides en volume.
Banyuls: 79 % (les diatomées sont plus communes ici).
Algérie W.: 95,4 %.
Sicile à Crète: 82,1 % (*Exuviella* fait ici une concurrence notable).

Les palmellas de *C. fragilis* font les 29/30 de ce volume de calcioflagellés dans le sud méditerranéen (Algérie, Tripolitaine), les $^2/_3$ ou les $^3/_4$ dans le nord méditerranéen (Monaco, Grèce) où les *Syracosphaera*, *Pontosphaera* ... sont plus nombreuses et variées. Je n'ai pas tenu compte des stades non palmelloïdes de *Coccolithus* (spores, kystes ...) car ces cellules, à pigment mélaniques très sombre, ne laissent pas deviner s'il s'agit de protistes encore vivants ou de cadavres, ramenés avec la vase du fond.

Les autres groupes font donc seulement 3 à 21 % du volume végétal moyen, mais pour la production réelle leur vitesse de multiplication, souvent plus grande que celle des *Coccolithus*, doit être considérée. Il n'est pas douteux que les organismes suivants jouent un rôle, peut être indispensable, dans l'alimentation des animaux, au moins par leurs vitamines.

En regardant le Tableau 1 on remarquera l'extrême abondance du *sable corrodé*, d'origine éolienne et fluviatile, au large de la Lybie (130 fois plus de grains par ml. qu'au large de Monaco, 7000 fois plus que près de l'Algérie et 2 fois plus qu'à Gibraltar). D'origine saharienne, ce sable doit gêner plus ou moins les organismes et se trouve, presque aussi abondamment, jusqu'au Cap Matapan. A notre avis, il explique en partie la pauvreté de la Méditerranée orientale en zooplancton.

2. Dinoflagellés

Dans le bassin occidental, leur volume ne représente que 3,5 à 4,5 % du total, grâce surtout à des *Ceratium* et à de petits gymnodiniens de 3 à 15 μ. Dans le sud du bassin oriental (Sicile à Crète, juillet 1955), cette proportion monte à plus de 17 %, à cause du pullulement d'*Exuviella marina* Cienkowsky. Cette thécatale pullule également à Tanger, d'après les récents travaux de G. Trotet. Pourquoi est-elle beaucoup plus abondante qu'ailleurs au large de Tanger et de la Tripolitaine? Elle devrait abonder aussi en Algérie,

Tableau 1
Comparaison des moyennes d'été de 0 à 2.500 mètres entre trois régions méditerranéennes

		Total du phyto-plancton par ml.	Palmellas de *Coccolithus*	Autres flagellés calcaires	Gymnodiniens	*Exuviella marina*	Petits flagellés nus	*Nostoc*	Grains de sable corrodé par ml.		Remarques diverses
Nombre d'éléments par ml.	M	109	9	10	5,6	0,3	18	60	13	7,8	Nombre de cellules vivantes par grain de sable
	Al	759	175	6	47	6,2	392	133	0,4	190	
	Cr	461	112	5	34,5	104	151	140	1766	0,26	
Volume moyen par élément (μ^3)			3200	1800	400	600	14	7			Moyennes calculées d'après les dimensions de 50 cellules différentes en divers mois
Volume total par ml. (décamicrons cubes)	M	50,7	29,2	18	2,07	0,19	0,25	0,4			Masse de *Coccolithus* probablement formée par mc d'eau en un an: 36 g. (Al.)
	Al	599,4	560	10,8	17,3	3,85	5,5	0,93			
	Cr	446,8	360	9	12,8	64,3	2,1	0,98			
% en volume dans le total général	M		57	35,5	4,1	0,37	0,5	0,8			Par ces %, le détroit de Gibraltar rappelle Monaco ou Banyuls, et le détroit de Sicile rappelle la moyenne de Sicile à la Crète.
	Al		93,6	1,8	2,9	0,64	0,9	0,16			
	Cr		80,1	2	2,9	14,4	0,47	0,22			

Légende du tableau: M = 6 à 12 milles au large de Monaco; de juin à septembre.
Al = 3 à 25 milles au large de l'Algérie W; de juin à août.
Cr = Sicile à Crète ("Calypso", juillet 1955).
Chaque valeur représente la moyenne de 1.000 prises d'eau (Al), 530 (Monaco), 170 (Cr).
Voir aussi la légende de la Figure 1.

située entre ces deux régions. Il y a sans doute là une question d'intolérance pour les forts changements de la salinité, qui, en Algérie, peut varier brusquement de 36,2 à 38,5 ‰, en raison du courant atlantique local. Quant à sa plus grande production dans le sud (en moyenne cent fois plus forte sur la ligne Tanger-Crète qu'à Banyuls et Monaco), elle s'explique aisément par l'insolation, les dinoflagellés se révélant beaucoup plus avides de lumière que les diatomées et les Coccolithophorides. Au large d'Alger (F. Bernard) et de Tanger (G. Trotet), chaque jour de vif éclairement solaire est suivi par une prépondérance nette des *Exuviella* et *Ceratium* sur les autres protistes de la couche euphotique. Mais les *Exuviella* peuvent aussi bien réussir en profondeur, et leur maximum de Sicile à la Crète se place dans la couche 250 à 800 mètres. Il y en a aussi beaucoup dans la Mer Morte (F. Bernard, 1957).

3. Petits flagellés nus

Très variées, ces cellules de 3 à 20 μ appartiennent selon les cas aux volvocales, au cryptomonadines, au chrysomonadines .... Elles sont indéterminable au faible grossissement du microscope inversé. Il y en a généralement des centaines par ml., mais, en raison de leur volume très faible, elles ne font que 0,5 à 1 % du volume général (exceptionnellement 10 à 20 % lors des maxima, au printemps et en automne).

4. Myxophyceae

Les cyanophycées sont représentées avant tout par un petit *Nostoc* en chaînes, dont chaque élément mesure de 2 à 5 μ. La biologie de ces schizophytes paraît très différente selon que l'on est à Monaco (où ils abondent après les maxima du plancton général) ou dans le sud méditerranéen (où ils peuvent pulluler en eaux plutôt pauvres, avec optimum à 25, 100 et 400 mètres). En tous cas, ils ne paraissent guère autotrophes et sont très intolérants vis-à-vis de la vase en suspension, comme Hentschel le signalait déjà dans l'Atlantique central. Leur volume ne fait guère plus de 0,2 à 0,8 % du total.

De tous les groupes étudiés ici, les myxophycées sont les plus difficiles à comprendre, leurs brusques prolifération à certains niveaux n'étant pas explicable par les facteurs connus. Il faudrait sans doute tenir compte de la richesse locale en matières organiques, donnée très importante mais non encore analysée dans nos recherches[1]). On assiste par exemple, au large de l'Algérie, à de courtes productions estivales de plus de 25.000 *Nostoc* ou *Dactyliococcopsis* par *centimètre cube* d'eau de mer.

5. Autres unicellulaires

Dans les numérations au microscope inversé, les autres groupes sont pratiquement négligeables, même en ce qui concerne les diatomées. Ces dernières ont encore une certaine abondance, surtout printanière, au large de Monaco et de Banyuls. Mais, vers l'Algérie et la Lybie, plus de 95 % de nos échantillons d'eau ne montrent aucune diatomée. Dans les 5 % restants, leur densité fait rarement plus de 30 cellules par ml., soit moins de $^1/_{20}$ du total des protistes, et l'optimum

[1]) Le procédé de Kay (Kiel, 1953) est en cours de mise au point à la Station Maritime d'Alger.

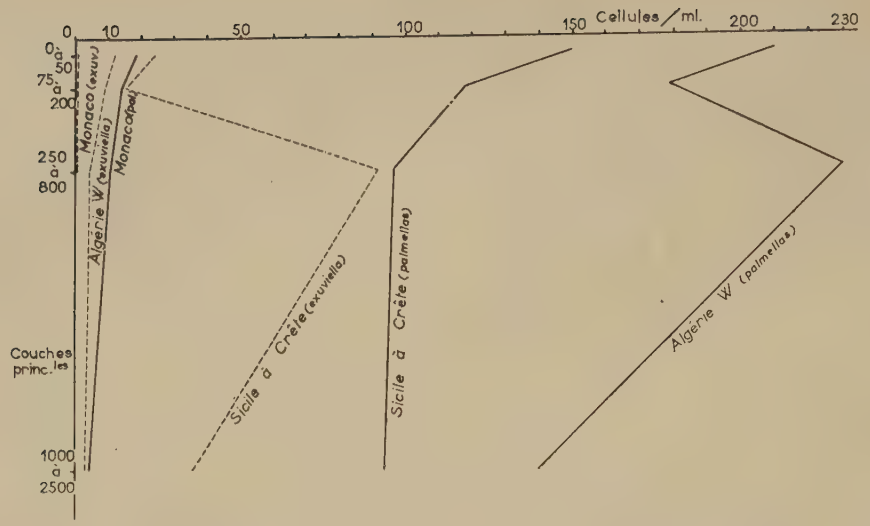

Figure 2. Nombres moyens de palmellas (cellules en division) de *Coccolithus fragilis* Lohm. par ml. d'eau (diagrammes en traits pleins) et d'*Exuviella marina* Cienkowsky (traits pointillés) pour les couches marines et les régions suivantes:—

0—50 m. Moyennes des prises d'eau à 0, 10, 25, 35 et 50 mètres. Cette couche est, en Méditerranée, la plus favorable à la photosynthèse et la plus constante de salinité au large de l'Algérie W., étant là presque toujours dans le courant atlantique.

75—200 m. Moyenne des prises à 75, 100, 150 et 200 mètres. Couche plus pauvre, et, au large de l'Algérie W., la plus variable de salinité, se trouvant tantôt dans le courant atlantique (36,1 à 37,0 ‰) tantôt dans la Méditerranée pure (37,6 à 38,6 ‰).

250—800 m. Moyenne des prises à 250, 300, 400, 500 et 800 mètres. Couche obscure, de salinité habituelle 37,8 à 38,9 ‰, et plus pauvre que la précédente à Monaco, plus riche en Algérie et en Tripolitaine où elle est fertilisée par le courant profond oriental.

1.000—2.500 m. Moyenne des prises à 1.000, 1.500, 2.000 et 2.500.

Monaco: moyenne des prises faites chaque semaine à 12 milles au large de Monaco, durant les années 1936 à 1938.

Algérie W.: moyenne de 13 coupes hydrologiques faites de 1 à 25 milles des côtes, d'Oran à Alger et Dellys, en été (juin-juillet) 1950 à 1952. La valeur pour chaque niveau résulte donc en général de la moyenne de 13 prises.

Sicile à Crète: moyenne de 10 stations entre Syracuse et le Cap Crio, exécutées par la "Calypso" en juillet 1955. On remarquera la richesse en *Exuviella*, surtout dans la couche 250—800, où elle est sans doute attribuable au profond courant oriental.

se place entre 50 et 150 m. de profondeur, au lieu de 0 à 50 m. à Monaco. Cela tient, non seulement à l'insolation accrue, mais aussi et surtout à la concurrence victorieuse des *Coccolithus* et des dinoflagellés, dont le niveau optimum dans la zone euphotique se place entre 0 et 50 mètres.

En résumé, l'écrasante prépondérance des flagellés calcaires dans la fertilité à tous niveaux apparaît encore mieux après les recherches récentes.

III. Variations de Fertilité selon les Principales Couches Marines

Dans la plupart de nos opérations en mer, les niveaux étudiés furent les suivants: 0, 10, 25, 35, 50, 75, 100, 200, 250, 300, 400, 500, 800, 1000, 1500, 2000 et 2500 mètres. Le détail des résultats a déjà paru, ou sera exposé plus tard. Pour la généralité biologique, il sera préférable de distinguer ici simplement les quatre grandes couches suivantes:—

0—50 m.

Optimum de photosynthèse pour les coccolithophorides et dinoflagellés. Couche la plus atteinte par les apports d'eau douce et par les changements de température, et la plus parcourue par le courant atlantique au large de l'Algérie ouest.

75—200 m.

Optimum des diatomées (en petit nombre) dans le sud méditerranéen. Minimum assez général des dinoflagellés, mais maxima locaux de flagellés nus et de *Nostoc*. En Algérie, couche la plus variable comme salinités et la plus pauvre en *Coccolithus*.

250—800 m.

Couche obscure, mais souvent parcourue par le courant oriental profond, ce qui amène des maxima de *Coccolithus* en Algérie (jusqu'à 4.200 palmellas/ml.) et des maxima d'*Exuviella* et de *Nostoc* entre la Sicile et la Crète. A cause peut-être de ces concurrents,

les petits flagellés nus sont souvent ici à leur minimum. Salinité généralement maximum, des eaux venant d'Egypte et de Syrie passant ici.

1000—2500 m.

Couche en moyenne d'un tiers plus pauvre que la précédente, à courants rares (sauf quelques stations au large de la Sicile et de la Tripolitaine, où se manifestent de forts courants locaux et des poussées de nannoplancton).

La Figure 2 représente les faits moyens pour les palmellas de *Coccolithus* (traits pleins) et pour *Exuviella marina* (pointillés). On y voit d'abord un gradient de variation vers le bas beaucoup plus accentué dans le sud qu'à Monaco. Deux raisons peuvent être invoquées pour rendre compte de ces différences:

1. En Algérie et en Tripolitaine, l'énergie solaire reçue est 2 à 5 fois supérieure à celle du nord méditerranéen. Rien d'étonnant dès lors à ce que la couche 0—50 soit plus productive qu'à Monaco, cela étant accru par l'extrême pauvreté en phosphates du golfe de Gênes.

2. Dans le sud, depuis la Syrie jusqu'à Gibraltar, se manifeste le contre-courant oriental, maximum de 290 à 450 mètres, d'où apogée des *Coccolithus* à ce niveau en Algérie, des *Exuviella* en Tripolitaine. Les données actuelles ne permettent pas encore de bien comprendre pourquoi *Exuviella*, assez rare en Algérie (Figure 1), est douze fois plus abondant de la Sicile à la Crète vers ces profondeurs. En tout cas, se divisant plus vite que les *Coccolithus*, les *Exuviella* empêchent ces derniers d'atteindre au large de la Lybie les densités remarquables (500 à 4.000 cellules par ml.) constatées dans le courant oriental au large de l'Algérie.

Pour la clarté du diagramme, les autres organismes n'ont pas été représentés. Ils sont d'ailleurs 10 à 20 fois moins importants que les précédents, si l'on considère leur masse par litre. Les définitions ci-dessus des 4 couches essentielles montrent des maxima de petits flagellés nus de 75 à 200 m. (sauf à Monaco), de *Nostoc* de 75 à 800 (ils ne restent abondants jusqu'à 2.000 qu'à Monaco).

IV. Vitesse de Multiplication des Etres Dominants

Si beaucoup de diatomées et de péridiniens ont pu être cultivés *in vitro*, on a jusqu'à présent échoué dans la culture des *Coccolithus*, flagellés prépondérants de mer chaude. Pour évaluer leur cadence normale de mitose, on est réduit à deux sortes de renseignements, à vrai dire nombreux, convergents et relativement solides:—

1. Lohmann (1903—1913), à Syracuse, trouve qu'en mer le nombre de flagellés calcaires par litre augmente au plus d'un tiers par jour. D. Kruger (1950), après 6 mois de numérations soignées, jour par jour, près du port d'Alger, conclut que le nombre de *Coccolithus* s'accroît d'environ $^1/_5$ par jour, parfois un peu plus. C. Bertoldo (1956) obtient à Alger des résultats du même ordre, chose instructive car entre temps l'eau de la baie d'Alger avait changé de nature: purement méditerranéenne jusqu'en 1954, elle est devenue *atlantique* (36,1 à 36,8 ‰) vers octobre 1955 et le demeure actuellement. Donc, le changement de salinité n'a guère modifié la cadence de division des *Coccolithus*, et même, soit dit en passant, l'enrichissement en phosphates par le courant atlantique (3 à 5 fois plus riche en P que la Méditerranée pure) n'a pas amené ici une fertilité en flagellés très accrue. Dans l'ensemble des eaux du large, d'Oran à Bougie, le courant atlantique ne contient que 2 à 5 % de *Coccolithus* en plus de la Méditerranée pure locale.

2. E. Steemann Nielsen (1952—54), au moyen du ^{14}C, démontre que certaines mers très riches en diatomées (courant de Benguela, eaux nordiques . . .) doublent en 24 heures leur teneur en C organique. Par contre, les océans tropicaux (Indo-Pacifique, Atlantique central . . .), à profondeur moyenne (50—100 m.) augmentent généralement leur C organique de 15 à 25 % en une journée. Or, dans ces régions et à ces niveaux, les protistes autotrophes dominants sont presque certainement des *Coccolithus*.

Conclusion

Les *Coccolithus* se divisent 4 à 6 fois moins rapidement que les diatomées et les volvocales classiques des mers froides. Nous avons démontré (loc. cit., 1956, p. 316) que, par suite sans doute de cette lenteur, l'utilisation des sels nutritifs par le plancton à flagellés d'Algérie est défectueuse. Une proportion des phosphates et nitrates variant de 2/3 à 13/15 selon les niveaux reste inemployée, même en zone euphotique, par les végétaux pélagiques présents. C'est la vraie cause de la faible fertilité méditerranéenne, car, en Algérie ouest au moins, la pauvreté habituelle en sels nutritifs ne peut être invoquée; le courant atlantique apporte des phosphates, et les eaux douces côtières donnent à la mer un stock abondant de nitrates.

En se basant sur une moyenne d'1/6 d'augmentation par jour des cellules vivantes, la production annuelle de *Coccolithus* par mètre cube d'eau, au large de l'Algérie, peut être estimée à une moyenne de 36 g., squelette calcaire compris, et davantage pour les couches favorables 0—50 m. (euphotique) et 250—800 m. (hétérotrophe).

Reste à savoir si les autres organismes communs (dinoflagellés . . .) n'ont pas une cadence de mitose plus satisfaisante. C'est bien possible pour *Exuviella marina*, dont le grand protistologue E. Chatton me disait en 1942 la multiplication intense en cultures, de + 13° à + 16°. D'après les travaux norvégiens, les grands péridiniens (*Ceratium*, *Gonyaulax*, *Peridinium* . . .) ne doublent pas en un jour comme les diatomées et sont intermédiaires à cet égard entre *Coccolithus* et *Exuviella*. Enfin, les *Nostoc* et petits flagellés nus doivent se diviser vite, mais ces deux

groupes, souvent hétérotrophes, ne jouent qu'un rôle secondaire en Méditerranée.

Brouardel et Rinck (1956) ont appliqué à 2 milles au sud de Monaco, en été 1955, la méthode danoise au ^{14}C, et trouvent 30 à 40 mg. de carbone assimilé par mètre carré de surface marine. Ces nombres ne représentent que le tiers des moyennes océaniques obtenues par Steemann Nielsen, mais sont encore trop rares pour conclure. Le sud méditerranéen (Algérie, Lybie) étant 9 à 12 fois plus riche en phytoplancton que le nord, il est donc permis de conclure que la fertilité dans cette région est égale ou supérieure à la moyenne des océans.

Summary

The real fertility in the Mediterranean is valuated from 0 to 2500 metres and from June to October. Three regions are compared: off Monaco (530 samples), off western Algeria (1000 samples), and between Sicily and Crete (170 samples).

Calcareous flagellates are prevailing by far at all levels (75 to 96 % of the phytoplankton volume per litre), the palmellae of *Coccolithus fragilis* Lohm. being the larger biomass. Dinoflagellates represent only 3 to 5 % of the total volume in the western basin, and more than 15 % off Libya, according to the local abundance of *Exuviella marina*. Naked flagellates and *Nostoc* make less than 1 % of the volume. In southern Mediterranean, diatoms are rare and practically without value.

The richest layers for *Coccolithus* are 0—50 m. (euphotic) and 250—800 m. (zone with oriental current). But the fertility does not decrease much with depth, and the layer 1000—2500 m. has yet $^2/_3$ of the density of cells observed from 250 to 800 m.

The stock of *Coccolithus* grows, at most, of about $^1/_5$ per day, and nutrient salts are very slowly used in the euphotic zone. However, the fertility in the southern Mediterranean seems equal or greater than in tropical oceans.

Between Sicily and Greece, the corroded sand, coming from Sahara, is very abundant (mean: 1766 grains per ml.) and probably troubles several organisms. The elementary fertility is, however, of the same order as off Algeria, where the coasts are protected from desert sand by mountains. The local Atlantic current makes the Algerian Sea richer in phosphates, but not practically richer in flagellates.

Références

(On trouvera dans les premiers mémoires cités (1951 et 1956) des références beaucoup plus nombreuses; on ne cite ici les travaux postérieurs à 1950).

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C. 9.

Surface Chlorophyll "A", Surface Primary Production, and Zooplankton Volumes in the Eastern Pacific Ocean

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Introduction

In the first section of this paper nearly all of the simultaneously observed surface chlorophyll *a*, surface primary production, and zooplankton volume data, regardless of differences in time of collection, have been summarized in an attempt to present a picture of the "productivity" of the north-eastern Pacific Ocean. These observations are related in a qualitative manner to the physical and chemical regime in these waters. In the second section an attempt is made to compute the daily organic carbon requirement of the zooplankton captured by the meter net in the upper 300 metres and to compare this with the daily production of organic carbon by the phytoplankton. Lastly, photosynthetic rates per unit chlorophyll *a* observed in sea-water samples are compared to similar rates measured in the laboratory by other investigators.

Geographical variations in surface chlorophyll *a*, surface primary production, and zooplankton volume

In Figures 1 and 2 the values of surface chlorophyll *a* and surface *in situ* primary production are presented. The data have been averaged over each 5° square of latitude and longitude, regardless of the date of sampling; the number of observations used in obtaining this average is given in the upper right hand corner of each square. The zooplankton volume data, presented in a somewhat different manner, are illustrated in Figure 3. In spite of the rather patchy series of observations, a few qualitative generalizations may be made about them and their relation to the physical and chemical environment.

Let us first consider the region offshore of the western United States and Mexico north of about 15° N. and extending northward to about 40° N. In this region we find low concentrations of chlorophyll *a* (0·100 mg./m³., or less), low surface primary production (less than approximately 2 mg.C/m³./day, with a number of individual values less than 0·50 mg. C/m³./day), and low zooplankton volumes in the upper 300 m. (generally less than 50 ml./1000 m³.). In the south-eastern portion of this area the mixed layer is low in soluble inorganic phosphate (about 0·3 μg.-at. PO_4–P/l.), and nitrate is present in trace quantities only. The water is likewise quite transparent, averaging greater than 96 % transmission per metre in the blue-green spectral region (480 $\pm$ 50 mμ). Scattered observations throughout the remaining portion of the area also indicate the existence of the same general conditions although phosphate-phosphorus concentrations appear somewhat greater to the north.

The region under consideration appears to lie in the eastern edge of a gyra discernible in the Sverdrup *et al.* (1942) current chart (Fig. 4). While the existence of this gyra cannot yet be said to be a permanent feature of the circulation in the north-eastern Pacific, evidence from different sources seems to support this idea. This gyra appeared from Munk's (1950) calculations, and the U.S. Hydrographic Office Atlas of surface currents in this region (H.O. No. 570) yields no information contrary to this supposition. This is also the area occupied by the atmospheric "high" in the north Pacific. This current (and biological) boundary likewise corresponds to the eastern edge of an extensive red clay deposit (see Sverdrup *et al.*, p. 972 and Fig. 253, p. 975). Although data are not available, it is tempting to suggest that the entire area of the gyra is likewise poor with respect to chlorophyll *a*, surface production, and zooplankton.

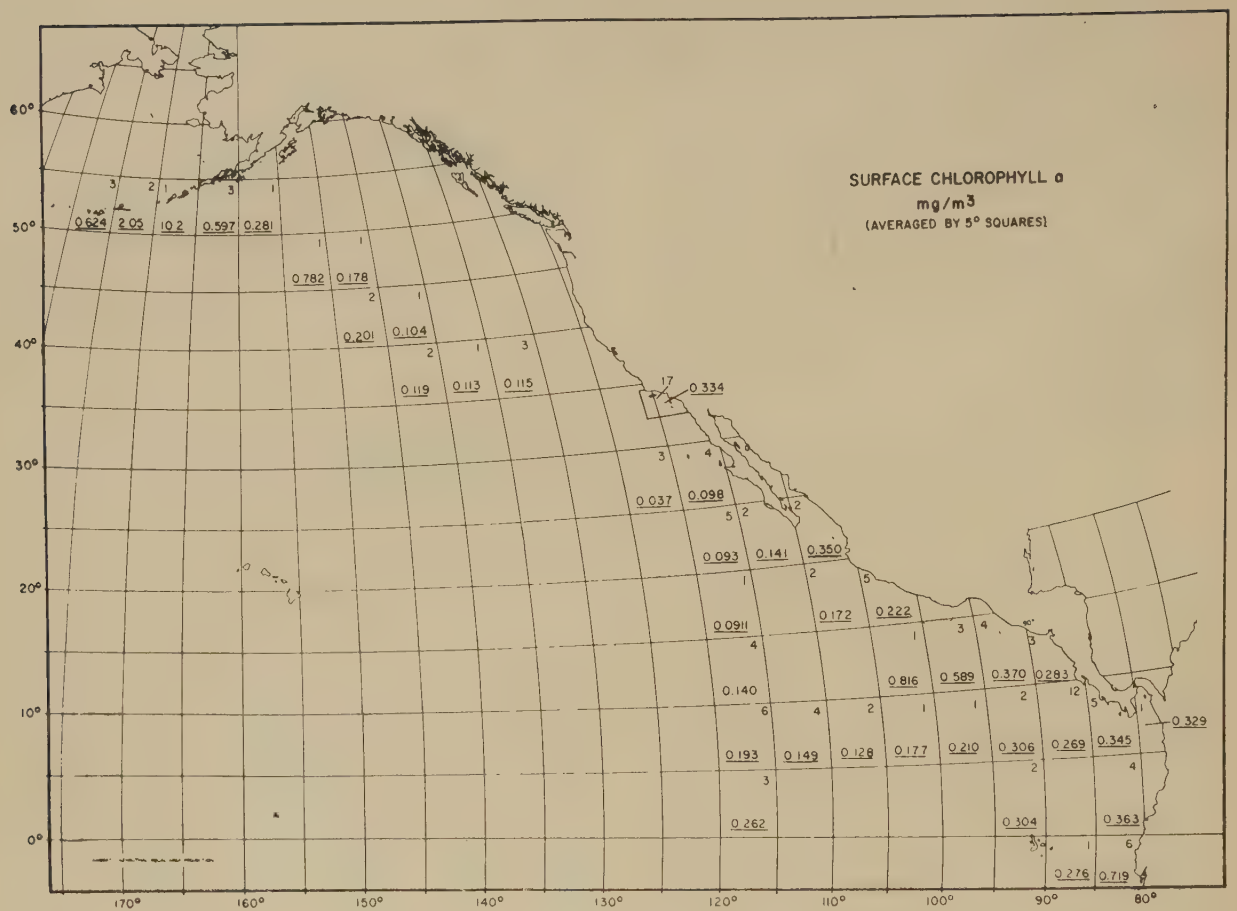

Figure 1.

Higher values of surface chlorophyll *a*, surface primary production, and zooplankton volumes are found in the region of the northern boundary of the Equatorial Countercurrent (*ca.* 10° N.) and these values trend upward from west to east toward the coast of Central America. This phenomenon seems to be related to the thermocline, which is elevated close to the surface as a ridge along this boundary, and which shoals from west to east. For example, in 1955 the mixed layer had a thickness of about 30 metres at about 115° W., while at about 90° W. it was only 18 metres thick. At the inshore end, this ridge appears to spread out into a broad "dome" off the Central American coast, the thermocline becoming slightly deeper again close inshore. In the region of this "dome" we find relatively high values of surface chlorophyll *a* (0·269 to 0·345 mg./m³.), surface primary production (9·0 to 20 mg.C/m³./day), and zooplankton volumes (200 ml./1000 m³. and higher). In 1956, phosphate-phosphorus concentrations were moderately high (0·7—1·0 μg.-at. PO_4-P/l.) and nitrate values, though quite variable, were generally higher than those encountered in the waters to the north-west. Water transparency was also moderately low, averaging 90—94 % transmission per metre in the blue-green spectral region (480 $\pm$ 50 mμ).

In the region of the northern boundary of the Peru Current, off northern South America and in the vicinity of the Galapagos Islands high surface chlorophyll *a* (0·719 mg./m³.), surface productivity (15 and 15 mg.C/m³./day) and zooplankton volumes (up to 600 ml./1000 m³.) are found.

Moderately high levels of chlorophyll *a*, primary production, and zooplankton volumes are shown for the South Equatorial Current in the vicinity of the equator near 115° W. longitude. Hydrographic data indicate the presence of upwelling along the equator (unpublished results).

Moderate to high values of cholorophyll *a*, production, and zooplankton volume appear in the area just off the coast of Mexico from the Gulf of Tehuantepec northward to the vicinity of Cape Corrientes. Nutrient concentrations are generally moderately high in the mixed layer in this region. Some of this is probably

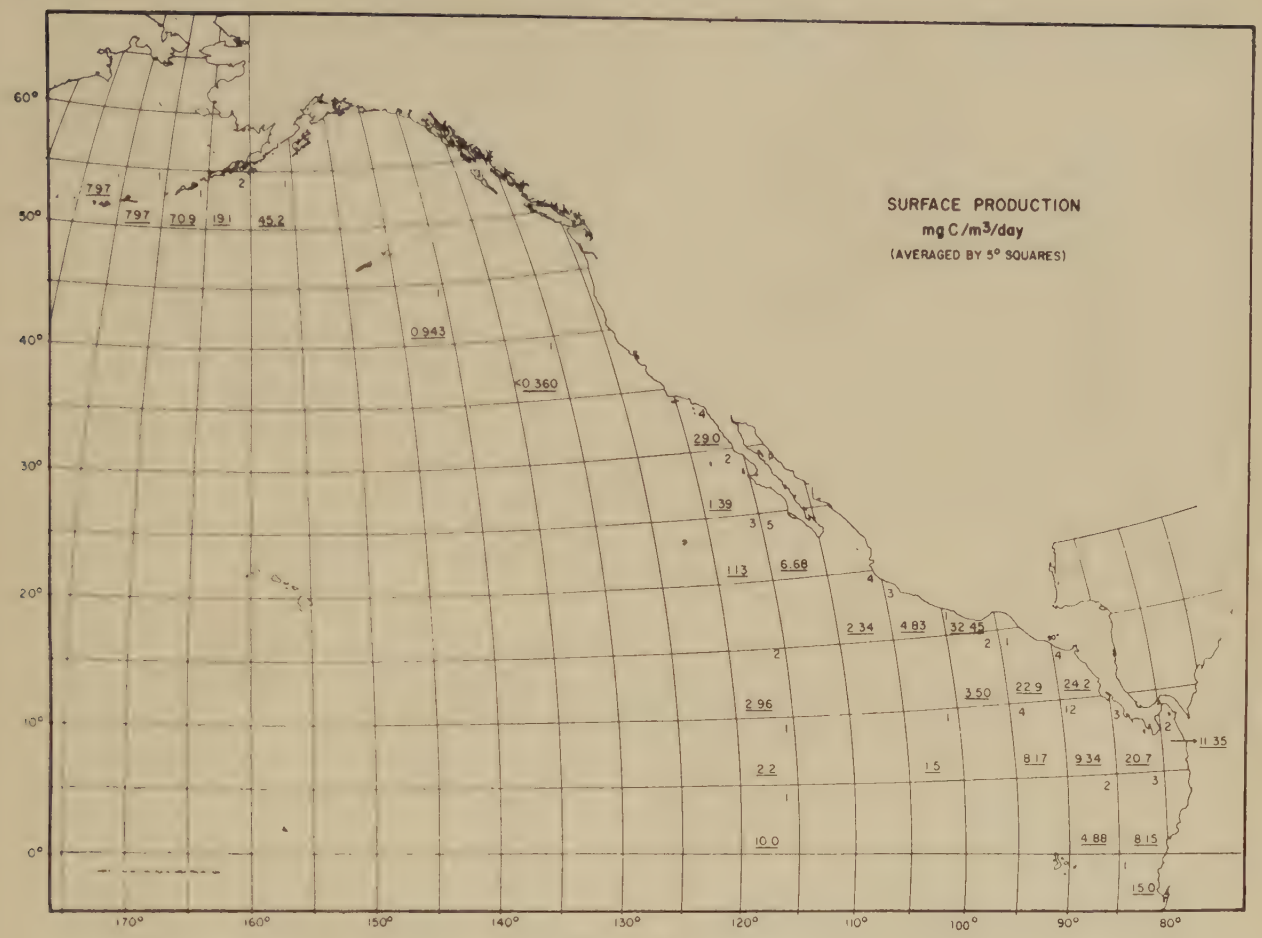

Figure 2.

the result of coastal upwelling, especially in the vicinity of the Gulf of Tehuantepec, but in general the causes of the biological activity cannot be said to be completely understood at present.

The Channel Island area off the coast of southern California seems to maintain about the same level of productivity (29 mg.C/m³./day) and surface chlorophyll *a* (0·334 mg./m³.) as the area off Central America, although there appears to be considerable spatial and temporal variation in these California waters. This area, like the Tehuantepec region, is one of upwelling. The upwelling is generally centered about Point Conception and is nearly a continuous phenomenon but with seasonal trends in magnitude.

The area lying just north and south of the Aleutian Islands, west of about 155° W. is quite rich (data from August 1953) and the phosphate-phosphorus concentration generally exceeds 1·10 μg.-at./l. in the mixed layer. The highest chlorophyll *a* and surface productivity values the author has observed in Pacific waters occurred in the Bering Sea (10 mg./m³. and 71 mg.C/m³./day, respectively). The causes for this richness at this time of year are not presently understood.

The general agreement between these three biological measurements is surprisingly good and in subtemperate and tropical waters any one will serve as a rough estimator of the other two.

The above observations, within the limits of the sampling programme, substantiate the predictions of Sverdrup (1955) which were largely based on physical oceanographic data. Deviations from the productivity pattern he predicted do occur but mostly involve the location of boundaries. Such differences may be the result of the incomplete coverage of the author's data. In general, agreement between predicted and observed productivity is remarkably good and supports Prof. Sverdrup's thesis that, other things being equal, productivity is closely related to the rate of replenishment of nutrients to the mixed layer.

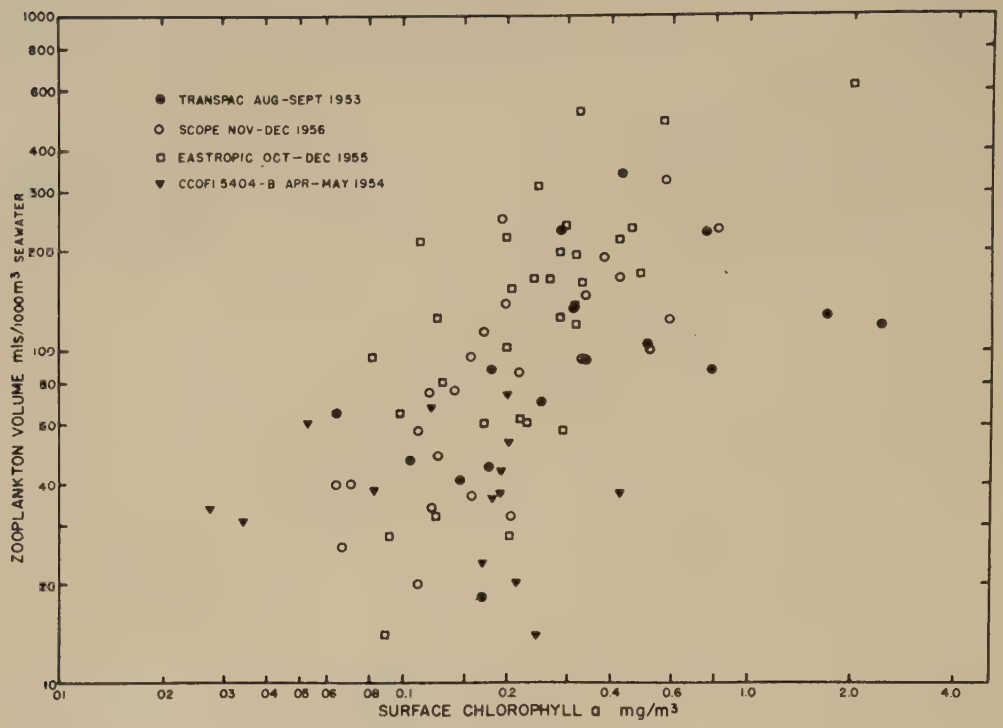

Figure 3.

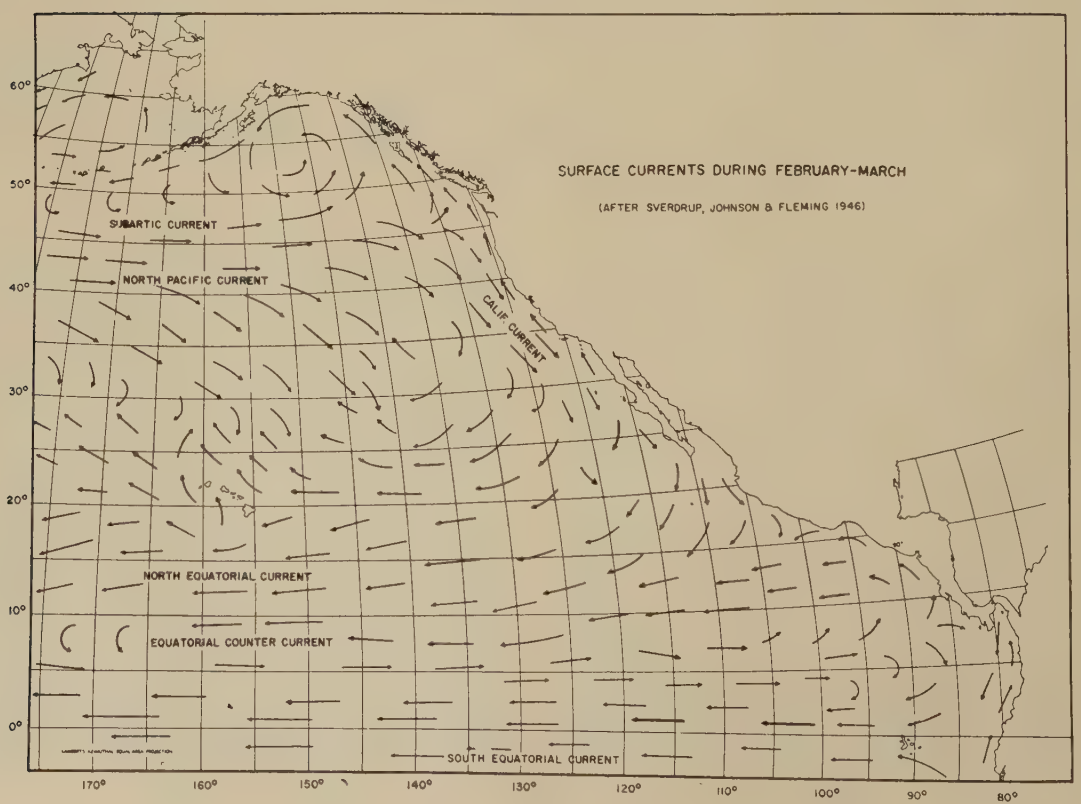

Figure 4.

Surface chlorophyll *a* — zooplankton relationships

In Figure 4 the zooplankton volume is presented as a function of surface chlorophyll *a* concentration for each station. While there is some scatter, the observations reveal that a relationship exists between these two quantities. The correlation might have been improved had we had available a sufficient number of vertical chlorophyll *a* profiles for integration and comparison with zooplankton volumes. Thus the variation seen in Figure 4 is considerably less than might have been expected—especially if certain observations from northern waters ("Transpac" data) are excluded. This lack of extreme variation indicates that in our southern waters we may well have a situation approaching a steady state. If we assume a steady state, the *in situ* water column productivity (g.C/m².day) minus the daily particulate organic carbon requirements of zooplankton not captured in the meter net, minus any particulate carbon metabolized by bacteria, minus the fallout, should equal the daily particulate organic carbon requirements of the zooplankton retained by the meter net.

During 1955 and 1956 the median value of *in situ* water column production observed at seven stations off Central America (see Table 1) was 0·442 g.C/m².day (range, 0·163—1·02); the median zooplankton volume in the upper 300 metres was 166 ml./1000 m³. (range, 161—250). If we assume a steady state, then 0·442 mg.C/m².day, less the above mentioned three unknown quantities, should equal the grazing rate. To obtain an estimate of the grazing rate in terms of organic carbon it has been necessary to use the results of laboratory feeding experiments. Dr. R. Lasker of the Scripps Institution of Oceanography has kindly provided me with the necessary information obtained in a recent series of experiments with *Euphausia pacifica*, as yet unpublished. Dr. Lasker reports the following:

(1) the wet weight of an adult *E. pacifica* averages 0·025 mg. (density assumed to be unity in calculations),
(2) the filtering rate in an adult averages 1·0 ml./hr. over a 24 hour period, and
(3) the median value for the amount of particulate organic carbon filtered by a starved adult *E. pacifica* is 0·05 mg./24 hours (range, 1·0—0·005).

If we convert the median zooplankton volume observed at the seven stations (see Table 1) to an equivalent volume of *E. pacifica* and compute the daily particulate organic carbon requirement of the number of individuals contained in this column, we obtain 0·385 mg.C. This requirement is 0·090 g. less than the median daily production. This excess is then available for fallout, etc., and represents some 20 % of the particulate carbon produced per day. Unfortunately we have no adequate estimates of the amount of zooplankton not captured by the net, nor of the bacterial requirements, nor of the fallout in these tropical waters, and this makes it impossible to determine how the remaining carbon is partitioned.

While these observations and calculations reveal that the daily production by the phytoplankton exceeds the demands of the zooplankton captured in the upper 300 metres, it is also apparent that the concentration per unit volume of particulate organic carbon must be considerably greater than that amount produced per day by the phytoplankton if the zooplankton are to survive. Based on a filtering rate of 1 ml. per hour, an adult *E. pacifica* would need a particulate organic carbon concentration of 2·1 mg./l. Fox *et al.* (1952) give the concentration of particulate organic carbon for a station in waters off San Diego: from 0·47—0·16 mg./l. in the depth range 100—300 metres and 5·0 mg./l. at or near the surface. If these values hold for tropical waters it would appear that the zooplankton cannot survive in the 100—300 m. depth range for any length of time and must migrate to the surface where food is available in sufficient quantity. If the zooplankton spend one half of each day in the upper layers, then the particulate carbon concentration would need to be twice the above figure, or 4·2 mg./l.—a value quite close to the average concentration for surface water given by Fox *et al.* (1952).

If 4·2 mg./l. may be considered a minimal value for the concentration of particulate organic carbon in the surface layer, and we believe this material to be of autochthonus photosynthetic origin, it is evident

Table 1

Date	Lat.	Long. °W.	*In situ* productivity g.C/m.²/day	Zooplankton volume ml./1000 m.³ (in the upper 300 m.)	Equiv. no. *E. pacifica* per 1000 m.³	Daily requirement of *E. pacifica* g.C/day	Excess (+) Deficit (—) mg.C/m.²/day
13 Oct. 1955 ...	0°04′ N.	115°39′	0·510	166	6640	0·352	+(0·158)
3 Nov. 1955 ...	10°39′ N.	89°51′	0·163	193	7720	0·409	—(0·246)
4 Nov. 1955 ...	9°27′ N.	87°53′	0·442	164	6560	0·338	+(0·104)
5 Nov. 1955 ...	7°33′ N.	87°21′	0·779	161	6440	0·341	+(0·438)
23 Nov. 1955 ...	4°03′ S.	84°11′	1·02	219	8760	0·464	+(0·736)
20 Nov. 1956 ...	9°08′ N.	89°16′	0·316	135	5400	0·270	+(0·046)
21 Nov. 1956 ...	9°33′ N.	89°13′	0·368	250	10000	0·500	—(0·138)
		Median	0·442	166	6640	0·352	+(0·090)
		Mean	0·513	184	7360	0·368	+(0·146)

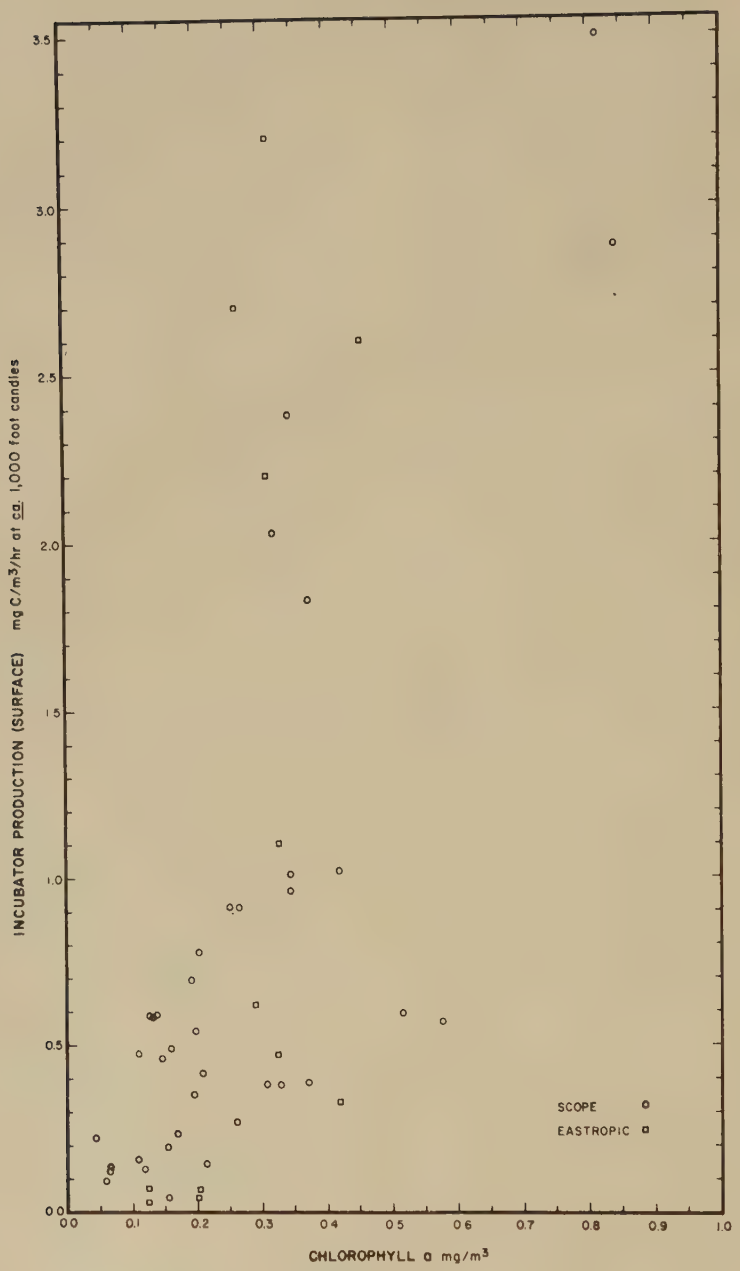

Figure 5.

that the daily production represents only a small fraction of the total carbon. In the waters off Costa Rica for example, the daily surface production averages 21 mg.C/m³./day, or only 0·05 % of the postulated carbon content of the surface water.

Productivity per unit chlorophyll *a* in surface waters

The relationship between chlorophyll *a* concentration and photosynthesis has been investigated and discussed by a number of people—see for instance

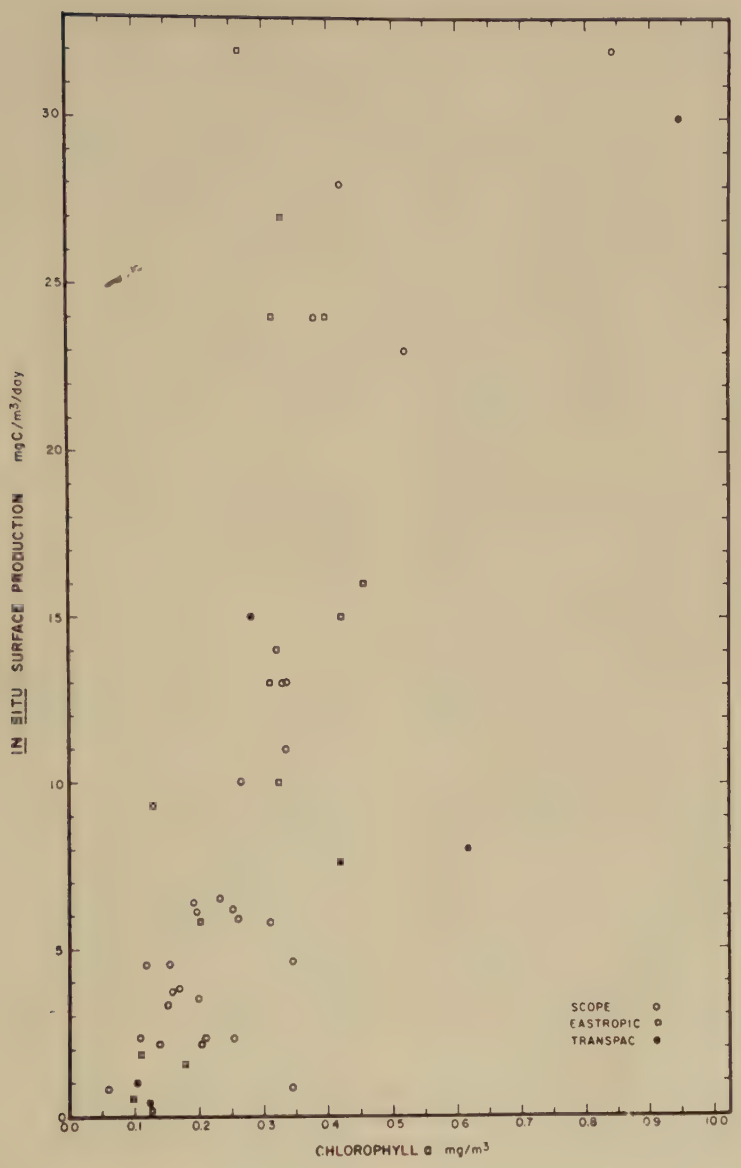

Figure 6.

Clendenning, *et al.*, 1956; Ryther, 1956; and Rabinowitch, 1951. Since most of these observations were obtained with laboratory cultures it should be interesting to compare productivity per unit chlorophyll *a* as observed in nature with that obtained under controlled conditions in the laboratory.

The data available for this comparison are of two types: (a) the photosynthetic rates obtained with surface samples incubated for four hours under constant light (1000 $\pm$ 40 foot candles) at sea surface temperatures and (b) the rates per day of surface samples trailed astern of the vessel for about six

hours (sunrise to noon, or noon to sunset). Chlorophyll *a* determinations were made from water samples collected at, or nearly at, the same time as the samples for the photosynthesis studies.

In Figure 5 the photosynthetic rates obtained with surface samples placed in the incubator and the corresponding chlorophyll *a* concentrations are illustrated. These data appear to fall into two groupings, each exhibiting different rates per unit chlorophyll *a*. Eye-fitted best fit lines drawn through these two groups of data yield the following average rates: 7+ mg.C/hr./mg. chlorophyll *a* for the data exhibiting the high rates (all data from the "dome" area off Central America) and 2 mg.C/hr./mg. chlorophyll *a* for the majority of observations. Assuming a photosynthetic quotient of 1·00, equivalent values in terms of oxygen production would be 13 and 3·7 ml. oxygen/hr. respectively, per mg. chlorophyll *a*. Clendenning *et al.* (1956) give rates on this same basis for a number of algae grown at 25° C. under conditions of light saturation, and compare their values with those obtained in higher plants and other algae by other investigators. These ranged from 2·64 to 14·77 ml. oxygen/hr./mg. chlorophyll *a*. The average rate observed by Ryther (1956) for 15 species of algae grown under undefined conditions was 22·7 ml. oxygen/hr./mg. chlorophyll *a*. At the depth of maximum photosynthesis in nine Wisconsin lakes, Manning and Juday (1941) found an average rate of 6·7 ml. oxygen/hr./mg. chlorophyll.

The average rate for the three stations which yielded the highest rate per unit chlorophyll *a* was nearly 20 ml. oxygen/hr. and is close to the average value given by Ryther (1956).

The six hour *in situ* ocean measurements (Fig. 6) yield an average rate somewhat higher than that obtained from the samples incubated on shipboard. Also the scatter of points is somewhat less with these *in situ* observations.

It is impossible to state why some of the rates observed in the incubator and *in situ* experiments are so low. The cause might be nutrient deficiencies and/or a large percentage of inactive chlorophyll *a*. But on the whole these data yield further confirmation of Ryther's suggestion (1956) that it should be possible to estimate productivity, at least at the surface, from the concentration of chlorophyll *a*, when the light saturation intensity for the population under consideration is known.

Appendix I

A multiple regression equation has been calculated using log-transformed "*Scope*" and "*Eastropic*" data. In all some 30 sets of observations were available and the following equation was obtained:—

$$\hat{Y} = 0{\cdot}161 + 0{\cdot}369\ X_1 + 0{\cdot}832\ X_2$$

where $\hat{Y}$ is the logarithm of the zooplankton volume in ml./1000 m³., X_1 the logarithm of the surface *in situ* productivity (mg.C/m³./day), and X_2 thelogarithm of surface clorophyll *a* concentration (mg./m.³). The standard error of the estimate is 0.0151 which corresponds to about 10 %. Both surface *in situ* production and surface clorophyll *a* may be employed individually to estimate zooplankton volume although the precision is not great (standard error: 17 % and 13 %, respectively).

Appendix II

If we exclude the data obtained in northern waters and consider only the "*Scope*" and "*Eastropic*" data, the regression for these is:—

$$\hat{Y} = 0{\cdot}584 + 1{\cdot}012\ X$$

where X is the logarithm of the surface clorophyll *a* concentration (mg./m³.) and $\hat{Y}$ is the logarithm of the zooplankton volume (ml./100 m³.). The standard error of the estimate is 0·226 which corresponds to 1·68 %.

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C. 10.

Estimates of Primary Production in Biogeographical Regionization of the Ocean

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Thanks to the work of Riley, Steemann Nielsen, and several other scientists bearing on the evaluation of primary production in the ocean, it was discovered that the value of primary production varies considerably from one region of the ocean to another. It was but natural to expect that primary production, which depends on solar radiation, abundance of biogenic material, quantity and composition of phytoplankton should be greatly different in different regions. Hence it can be used to characterize these regions. At present the delimitation of biogeographical regions in the ocean, is based mainly on the distribution of individual representatives or complexes of fauna and flora. Such a zoogeographical and phytogeographical regionization is of great importance and has prospects of further development. However, it is also necessary to take into account the processes going on in the ocean. In this aspect, the assessing of primary production acquires a major importance. An attempt at such a delimitation of biogeographic regions was made by the Institute of Oceanology of the USSR Academy of Sciences. It was based on material from the north-western part of the Pacific, collected by the R/V "Vitiaz" during its cruises in the region south of the Kurile Islands.

The waters surveyed in this region, up to 30° N.L. were temperate boreal waters and northern branches of tropical Kuroshio waters. A comparison of their different characteristics permits to achieve a complex biogeographical evaluation of the boreal and the tropical regions of the north-western Pacific. The frontier dividing these regions lies near 40° N.L. The surface waters between the Kurile Islands and this boundary have usually a winter temperature below 3° C., increasing in summer to 14—15°. Southwards of the frontier, tropical Kuroshio waters predominate, with a surface temperature of 18—20° in winter and 26—28° in summer (see Fig. 1).

The specific composition of plankton in these regions is very different. North of 40° N.L. the plankton of the surface layers consists mainly of typical boreal species, as, for instance: *Thalassiosira nordenskiöldii*, *Chaetoceros convolutus*, *Ch. atlanticus*, *Ceratium longipes*, *Calanus plumchrus*, *Eucalanus bungii*, *Calanus cristatus* and other typical forms from the northern part of the Pacific Ocean.

To the south of 40° and, especially of 35° N.L., the tropical species in the plankton are predominant, such as: *Rhizosolenia bergonii*, *Chaetoceros lorenzianus*, *Climacodium biconcavum*, many species of Coccolitophora, *Cestus amphitrites*, *Velella*, *Physalia*, Heteropoda, Pteropoda, many species of Copepoda (*Acrocalanus*, *Undinula*, *Copilia*, *Sapphirina*, and others), *Halobates*, *Salpa*, *Pyrosoma*, and many others.

The biomass of plankton (weight per 1 m^3.) varies widely. Along the Kurile Islands there is a region with a medium value of biomass, ranging from 200 to 500 mg. per 1 m^3. Then follows a stretch, 30—50 miles wide, extending over more than 1000 miles, with a permanently high biomass, usually exceeding 500 mg. per m^3. Farther southwards lies a region with a diminished biomass. The warm waters of the Kuroshio are generally characterized by a low biomass of plankton, usually below 100 mg. per 1 m^3., often even less than 20 mg. per 1 m^3. Estimates of primary production carried out by the author together with C. W. Beklemishev (1955) show (see Fig. 2) that the diurnal production of carbon in the vicinity of the Kurile Islands does not exceed 0·1 mg. per 1 litre. In the zone of high biomass the average value of diurnal carbon production is almost three times greater, amounting to about 0·3 mg./l. Farther south the production of carbon decreases. In the region of warm Kuroshio waters it is 10 times lower than in the zone of intensive plankton development. On an average the production of carbon and the biomass

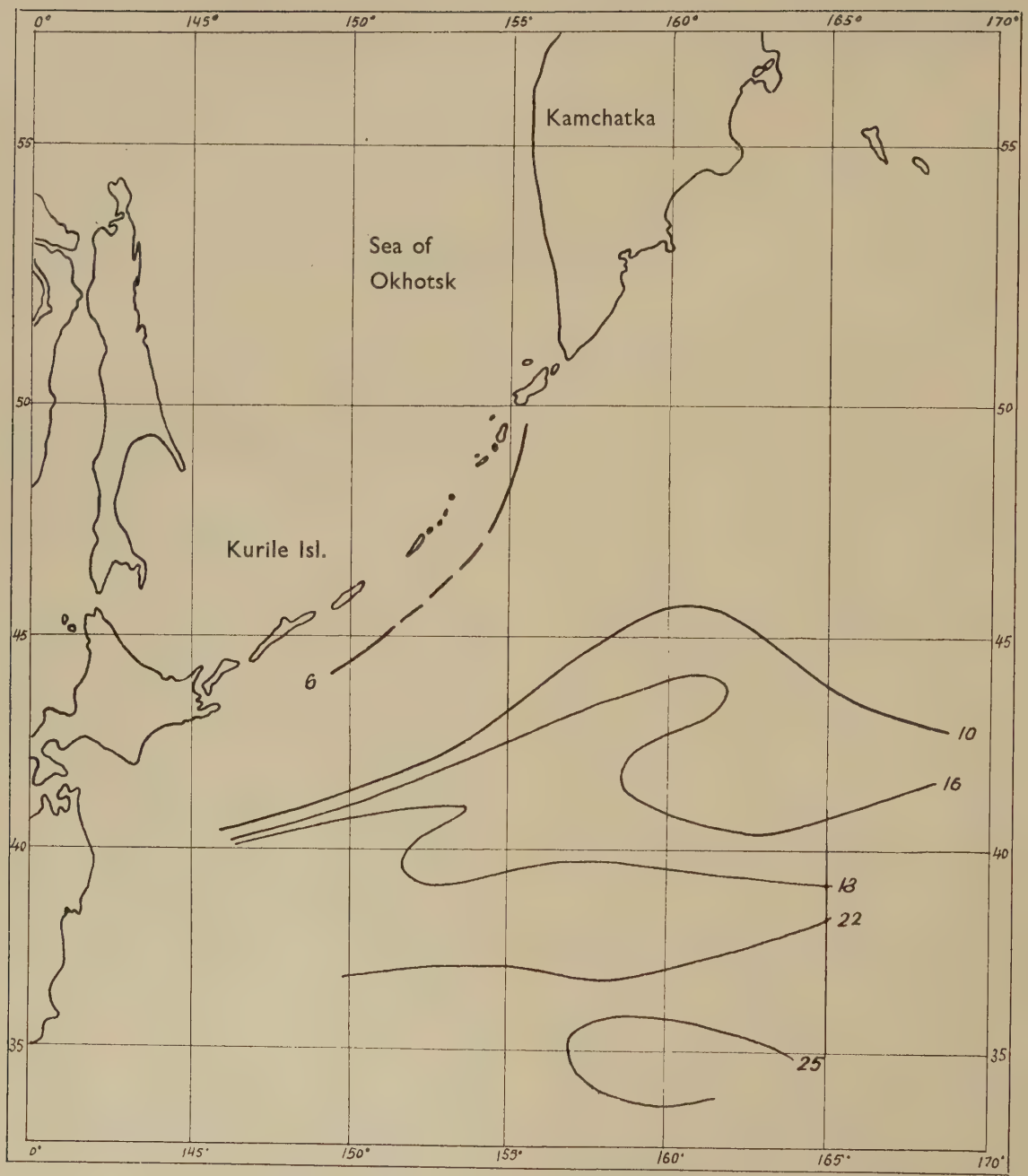

Figure 1. Surface temperature, early autumn 1954.

Figure 2. Average diurnal production of carbon in mg. per 1 litre in different parts of the north-western Pacific. Early autumn 1954 (determined by the oxygen method).

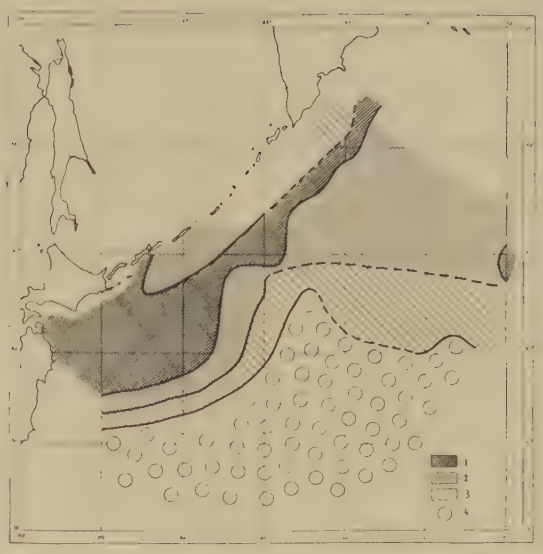

Figure 3. Biomass of zooplankton in the 0—100 m. layer. Early autumn 1954.
1 — over 500 mg./m.³ 2 — 250—500 mg./m.³
3 — 100—250 mg./m.³ 4 — less than 100 mg./m.³

of plankton in boreal waters are about tenfold as compared with those of the tropical waters. The impoverishment of the plankton biomass observed with the passage from the boreal into the tropical region is determined, first of all, by less favourable conditions for the creation of primary production, (such as mixing of waters, dynamics of nutrients for phytoplankton, etc.). The decrease of the plankton biomass results in a diminished food supply for fishes and whales.

It is also obvious that the primary production determines directly and especially indirectly the food supply not only in the surface layer but through the whole thickness of the ocean water masses. A quantitative analysis of plankton in different waters (see Fig. 3), down to the bottom, carried out by the author together with M. E. Vinogradov (1955), showed that the biomass of plankton from the surface to the floor of the ocean is higher in the boreal region than in the tropical. Furthermore the amount of organic matter in the ground and of silica in the bottom deposits is also less in tropical regions as compared with regions where boreal waters are prevailing. This is well demonstrated by a map drawn by P. L. Besrukov showing the distribution of amorphous silica in bottom deposits (see Fig. 4). This silica is produced by diatoms in the upper photosynthetic zone. A comparison of this map with maps showing the distribution of plankton biomass and primary production reveals an extraordinary coincidence of the essential features characterizing the distribution of the biomass of plankton and the distribution of silica in sediments[1]). This proves that the peculiarities of primary production in the upper layers of different oceanic regions affect the whole thickness of oceanic waters, and, hence, also the character of the bottom sediments.

These comparisons are meant to show the importance of estimates of primary production for the delimitation of biogeographical regions; they must be regarded merely as a first approach to a complex characteristic of the various regions of the ocean.

Investigations bearing on the primary production of the biogeographic regions of the World Ocean should, therefore, be developed as rapidly as possible. Since many methods are used in the determination of primary production, it will be necessary to reach an agreement about a universal method to be employed in mass analyses, that should be obligatory for scientists of different countries.

As known, two methods are widely used in the studies of primary production. The first consists in registering changes occurring in the oxygen content (Riley, 1938; Harvey, 1945), the other makes use of ^{14}C (Steemann Nielsen, 1952).

The oxygen method most nearly answers the purpose. It allows to determine separately the three values most relevant to marine biology, i. e., net production, gross production, and respiration. It is, however, not always applicable, owing to its low sensitivity. Thus it cannot be used in waters with a low productivity (for instance in the tropics) or, if used, requires extremely prolonged expositions.

The isotope method is employed to determine an intermediate value between "gross" and "net" production. This method has the great advantage of high sentivity so that brief expositions are possible. This permits to use the isotope method in tropical

[1]) The limits of plankton distribution are subject to some changes according to seasons and years, but the distribution of bottom sediments is more permanent. It reflects the process of life going on in all water masses from the surface to the bottom over very long periods of time.

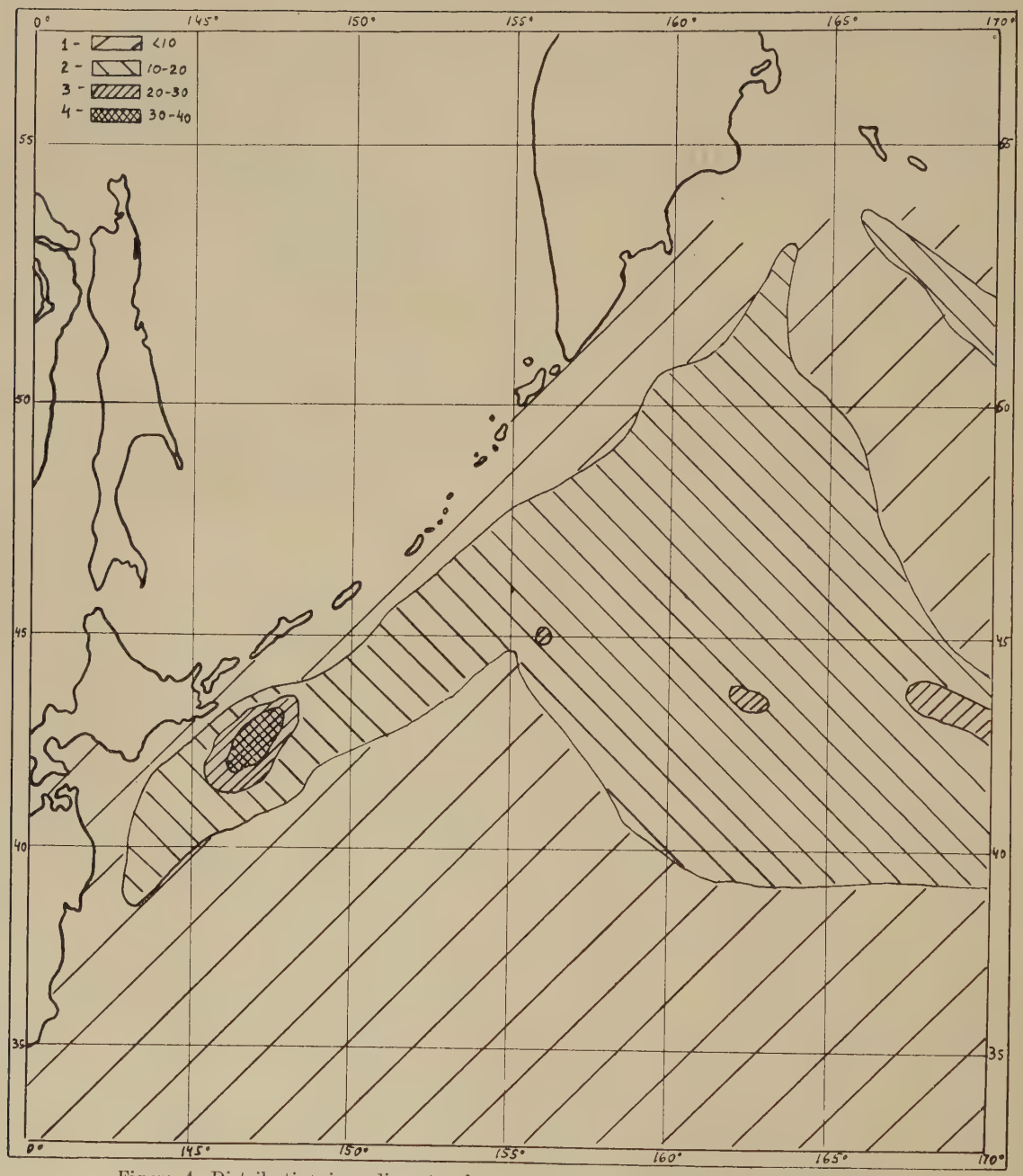

Figure 4. Distribution in sediments of amorphous silica, produced by diatoms (‰).

and other waters with a low content of phytoplankton. The method has also the additional advantage of easy manipulation and rapidity of determinations. Consequently the isotope method should be used in the future for mass collections of material, but everywhere and every time, when possible, determinations should be made by the oxygen method too in order to obtain comparable material.

The use of standard artificial illumination which, according to STEEMANN NIELSEN, enhances the comparability of the results, is as yet quite disputable. The results obtained with standard light are no more comparable than those obtained by exposition to natural light on deck. It is most probable that many algae are able to adapt themselves to their usual conditions of light.

Notwithstanding these critical remarks the problem of exposure of bottles to light is quite important. Pending its ultimate solution it seems advisable to expose the bottles directly in the water layers, from where the sample was collected.

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C. 11.

The Primary Production in Lakes: some Results and Restrictions of the ^{14}C Method

By Wilhelm Rodhe
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Introduction

The ^{14}C method introduced by Steemann Nielsen (1952, 1954) for the direct measurement of primary production in the sea offers an outstanding tool also for the determination of the CO_2 assimilation in the free water of lakes. Since June 1953 this technique, with slight modifications, has been used intensively at the Limnological Institute of the University, Uppsala, and its laboratory at Lake Erken. Thanks to its high sensitivity and the comparatively simple procedure, the ^{14}C method within the near future certainly will be regarded as indispensable for all laboratories where the productivity of natural waters is studied. Though the method in itself could well be developed into a routine procedure, the interpretation of its results at present involves several pitfalls and difficulties which may restrict and hamper its wider use.

It is the aim of this paper to summarize some main results obtained with the ^{14}C method in Swedish and a few other European lakes and to discuss, at the same time, the inherent inadequacy of these and similar results. The problems raised by this inadequacy seem, to a large extent, to be common for ^{14}C studies in all natural waters, salt as well as fresh, and should, therefore, have some bearing also on the topic of this conference.

Some of the following results have been presented at the symposium on "Perspectives in Marine Biology" at La Jolla and at the International Congress of Limnology in Helsinki and will appear in the proceedings of these conferences (Rodhe, Vollenweider, and Nauwerck, 1958, and Rodhe, 1958). A more detailed account of the present results is in preparation. Full and thankful credit is given to Dr. R. A. Vollenweider for kind permission to publish his excellent diagram given as Figure 1.

Vertical distribution

The vertical distribution of primary production is governed, in the first place, by the photic conditions prevailing in the water. The relationship between the photosynthesis of the phytoplankton and the vertical gradient of the light is, however, not a simple one. In the surface layer, down to a depth which depends on the light transmission (approximately: the transparency of the water), the light intensity usually is higher than the optimum for maximal assimilation rate of phytoplankton. Below the layer of optimal light, on the other hand, the lack of light is limiting this rate which therefore slows down to zero at successively greater depths. Thus the vertical curve for the primary production of organic matter in its typical form, as emphasized by Steemann Nielsen, is an optimum curve. Only on cloudy and dark days, and in the earliest and latest hours of clear days, the assimilation has its maximum at the very surface (Fig. 1).

For the performance of the ^{14}C experiments and for the interpretation of their results, it is essential that the inhibitory effect of superoptimal light intensities is affected by the length of exposure time: the longer the exposure, the greater the depression of the assimilation. Consequently, as shown in Figure 1, the sum of the results from consecutive short-time experiments (I—V) is greater than the corresponding result from a simultaneous series covering the whole day (VI). To use the example given in Figure 1, the data given in Table 1 hold for the dots (results of ^{14}C bottles *in situ*) of the summarizing diagram in the lower left corner.

Apparently the effect of longer exposure is greatest at the highest light intensity in the uppermost layer, where the sum obtained from five short-time exposures is about three times higher than the result from a

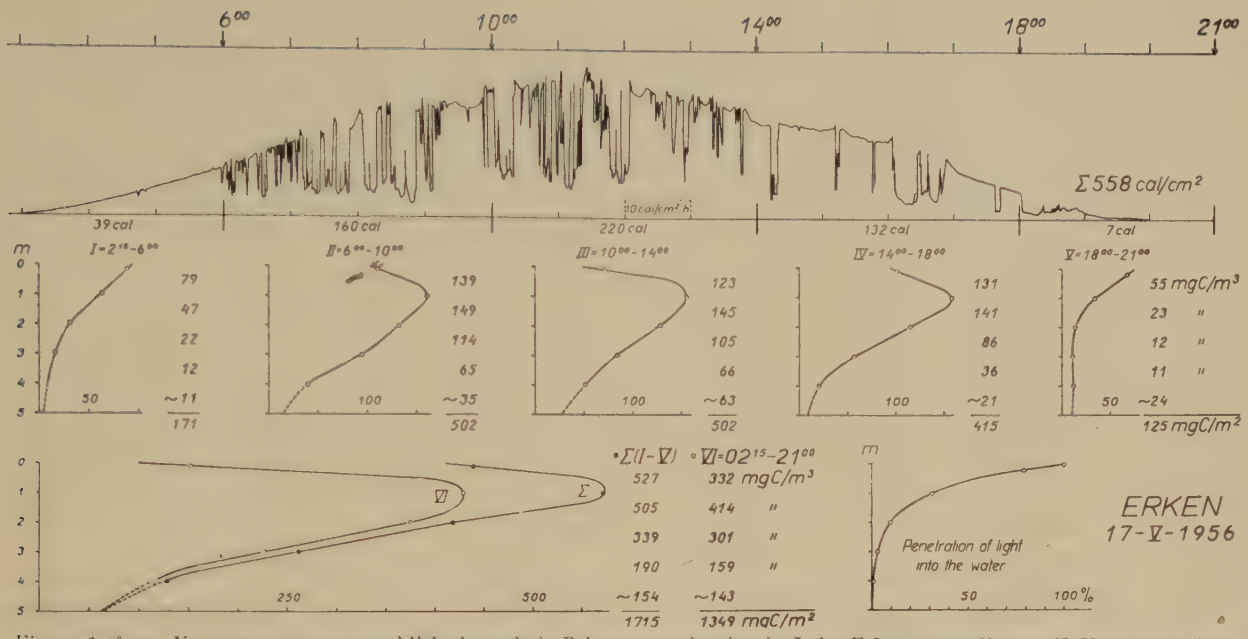

Figure 1 (from VOLLENWEIDER, unpublished results). Primary production in Lake Erken, according to (I–V) consecutive 4-hours series and (VI) a simultaneous whole-day series. Incident radiation of total energy in the top diagram, penetration of visible light into the water in the lower right corner.

bottle, exposed the whole day. Below the light optimum (from 2 to 4 m.) the differences of 12—15 % probably are caused mainly by decreasing activity of the algae enclosed in the long-time bottles for 19 hours. Calculated for the whole euphotic zone, the long-time series yields a value about 21 % lower than the sum of the short-time experiments (1349 versus 1715 mg.C/m², cfr. Fig. 1).

These results imply that we hardly are able to measure exactly the real assimilation in the free water by means of suspending ^{14}C bottles at various depths. Whereas the algae inside the upper bottles are exposed to high light intensities during several hours or almost a whole day—and, consequently, are subject to an exaggerated light inhibition—the currents and turbulence of the water constantly are changing the position and light exposition of the free-floating algae. Thus the actual primary production of the water column can be expected to be higher than the value derived from *in situ* experiments. This error is less on cloudy than on clear days, and it should be more important in turbid or coloured waters than in transparent water where a displacement of the algae involves smaller changes of light intensity. Further studies are needed to clarify these questions.

Anyhow, it seems appropriate to standardize, as far as possible, the length of exposure time for ^{14}C experiments *in situ*, intended to give comparable results. The author usually starts his ^{14}C series in lakes shortly after sunset and finishes them at sunset the next day. The best alternative is to run half-day experiments and to double their results in order to get figures for the whole day. These figures, however, generally will be somewhat higher than the whole-day records because of less light inhibition—apart from the random error caused by unequal insolation before and after noon. Experimental times shorter than half the sun-day are unsuitable for comparative purposes due to the difficulty to extrapolate their results into one-day figures. To run consecutive short-time experiments similar to those demonstrated in Figure 1, involves such an effort that they can be done only casually, for special purposes.

Table 1

Depth m.	From sunrise to sunset Σ (I–V) mg. C/m.³ × day	VI mg. C/m.³ × day	$\frac{\Sigma(I-V)}{VI} \times 100$ %
0·2	440	152	292
1	571	429	133
2	419	375	112
3	262	227	115
4	129	115	112

Seasonal succession

It certainly holds for every non-tropical, possibly also for many tropical waters that the amount of carbon daily assimilated per unit surface must be subject to considerable temporal variations. We still know very little, if anything, of the seasonal succession of primary production in various types of lakes, but

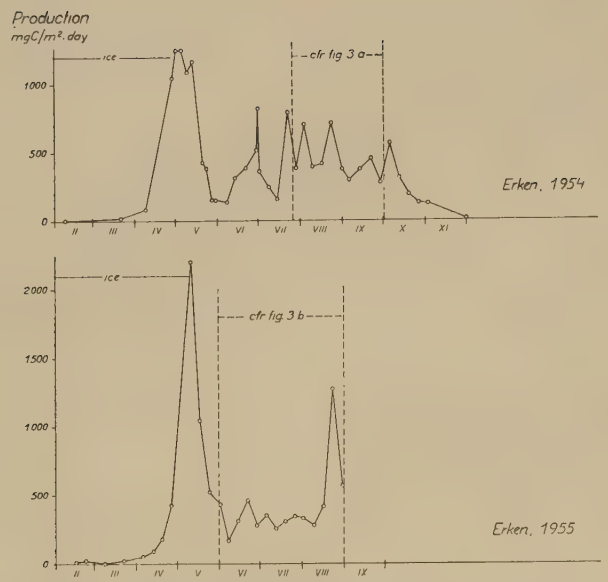

Figure 2. Seasonal succession of primary production per unit surface in Lake Erken, according to weekly or less frequent ^{14}C series *in situ*.

some results from the rather productive ("eutrophic") Lake Erken may be instructive, and useful when similar investigations are planned in other waters.

In Erken the primary production was measured at a station in the eastern part of the lake, with one 24-hours series every week from spring to autumn, less frequently in winter. The data obtained for the daily production/m². yield the diagram in Figure 2, where the dots in the usual fashion are connected by straight lines. A distinct modus of seasonal succession is evident: the maximum of the year in spring, starting already before the ice breaks and rapidly declining in May, a minimum in June and (1955) July, and then higher production again in August and (1954) September. In addition to this fundamental periodicity there are, however, several marked peaks during the summer and autumn which easily could be interpreted as short-term maxima of the temporal succession.

More detailed studies with daily, in addition to weekly data, have proved this interpretation often to be inadequate. The histograms of Figure 3 demonstrate that the production may vary considerably from one day to the next, particularly in August when these rapid changes up and down sometimes amount to several 100 %. At the beginning of summer (June, 1955), on the other hand, a more regular trend from day to day seems to be the rule. This behaviour of the short-time variations of production is correlated not with changes of the daily insolation but with the quantity and composition of the phytoplankton standing crop and its horizontal distribution in the lake. In early summer the phytoplankton mainly consists of small forms, nannoplankton, and at that time it is rather evenly distributed over the whole lake. In July and still more in August, the "netplankters" (in the first place blue-green algae and *Ceratium hirundinella*) have their best season and then a spatial heterogeneity evolves. Due to this patchiness of phytoplankton distribution, the wind gets a dominant influence upon the local conditions. In Erken, the shallow western part regularly has a higher standing crop of netplankters than the eastern part, and when western or northern winds push surface water towards the sampling station offshore the laboratory, the phytoplankton quantity, and with it, the primary production at once shows higher values. The contrary usually holds for eastern and southern winds, which bring poorer metalimnic water to the sampling station. In accordance herewith we state (Figure 3) that in most cases increases of the primary production coincide with western and northern winds and decreases with eastern and southern winds (these cases are indicated by a cross in the diagram).

The conclusion that can be drawn from this example is that a sampling frequency as high as once a week need not warrant a true picture of temporal succession. If the horizontal distribution is irregular, the water movements raised by the wind cause rapid changes which have very little in common with the successive development within a homogeneous water body. Under such circumstances, we get *one* picture on the basis of samples taken every Monday, but perhaps should achieve quite different peaks and feet in a curve derived from samples taken on Tuesdays, etc. The ^{14}C method in itself delivers reproducible results, the question is however, how representative they are. Straight lines connecting weekly dots as in Figure 2 cannot be regarded, *a priori*, as representative for the course of events during the other six days of the week, neither for the sampling station nor for the lake as a whole. In general, therefore, when we have to illustrate the seasonal succession, histograms are preferable to smoothed or broken curves which easily give an impression of more complete information than the primary results permit.

It will certainly be objected against this standpoint that horizontal irregularities of phytoplankton distribution, which are the main cause of rapid changes in primary production at a given station, may be much less pronounced in many other waters than in Erken. This is true, but it is a remarkable fact that as soon as sufficiently detailed studies are made, they reveal patchiness rather than uniformity to be the rule. Margalef (1958) recently discussed the genesis of this distribution pattern for a marine area. In any case the extent of possible daily variations should be investigated, before the significance of less frequent data is taken for granted.

Relations to standing crop of phytoplankton

The assimilation of netplankters and nannoplankters, respectively, can easily be determined by "frac-

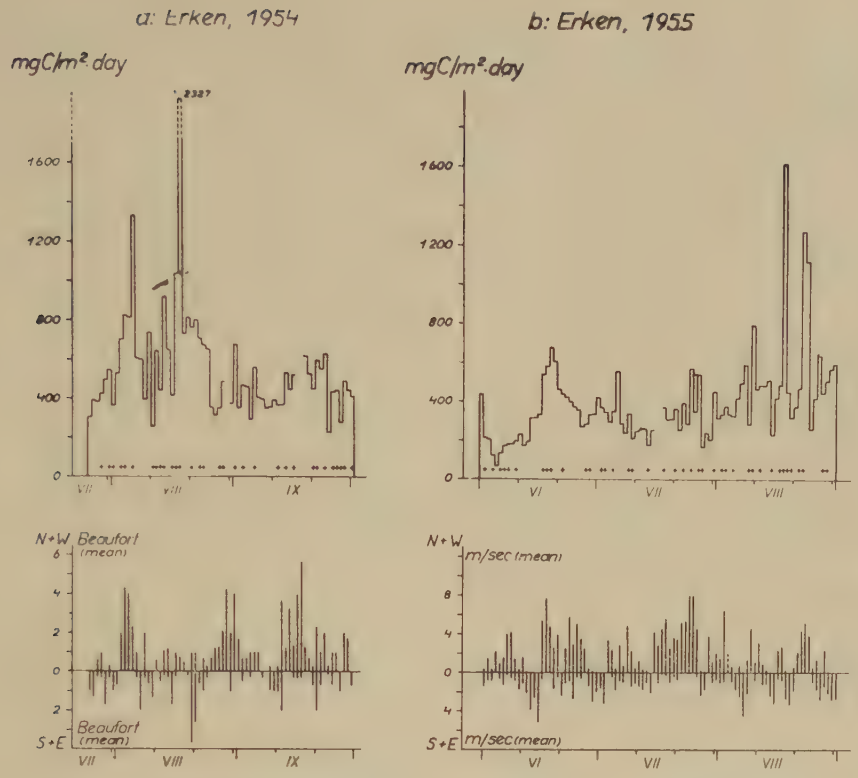

Figure 3. Seasonal succession of primary production per unit surface in Lake Erken, according to daily ^{14}C series *in situ*.

tionated filtration". An aliquot portion of the exposed ^{14}C sample is filtered at first through finest silk bolting cloth (Möllergaze No. 25) and then through membrane filter. The results are expressed as percentages of the total assimilation, which is obtained as usual by direct filtration of another aliquot portion through membrane filter only. In general, the sum of netplankton and nannoplankton assimilation is equal to 100 % within about ± 10 %.

For all lakes so far investigated with fractionated ^{14}C filtration it has proved to be the rule that the contribution of nannoplankton algae to primary production is greater than that of netplankton algae. Only occasionally, as during luxurious waterblooms, algae catchable with the finest plankton net perform more than 50 % of total assimilation. In most cases their contribution does not amount to more than 10—20 %.

Since the work of LOHMANN 50 years ago it is a well established fact—still disregarded by many students—that the nannoplankton often forms a major part of the phytoplankton standing crop. This is demonstrated in the example given in Figure 4, where the standing crop percentages are based on volume data, calculated as the sum of specimen volume × frequency for all species involved; the limit between netplankton and nannoplankton has been set at 100 μ, since the size of the finest plankton net meshes is about 70 μ.

It is evident from Figure 4 that the rôle of nannoplankters is even greater in primary production than it is in the standing crop. This means that the smaller algae, in general, are more effective producers of organic matter per unit volume than the netplankters are. The variable specific activity (assimilation per unit volume or unit fresh weight) of different algae is one of the reasons that we hardly can expect to find a general and constant relationship between the total standing crop and its assimilation rate. Only for unitary periods such as the spring outburst a linear relation was established between the primary production and the chlorophyll content in Lake Erken (RODHE, VOLLENWEIDER, and NAUWERCK, 1957).

Relations to chemical conditions

Though the supply of nutrients evidently must be essential for the extent of primary production, the relationship often is all but clear-cut if we compare the ^{14}C data with simultaneous results of chemical analyses. It happens, in particular, that high assimilation rates coincide with low contents of phosphorus and nitrogen in the water. This may simply be due to the exhaustion of nutrients because of their rapid consumption by the algae, but it remains to explain

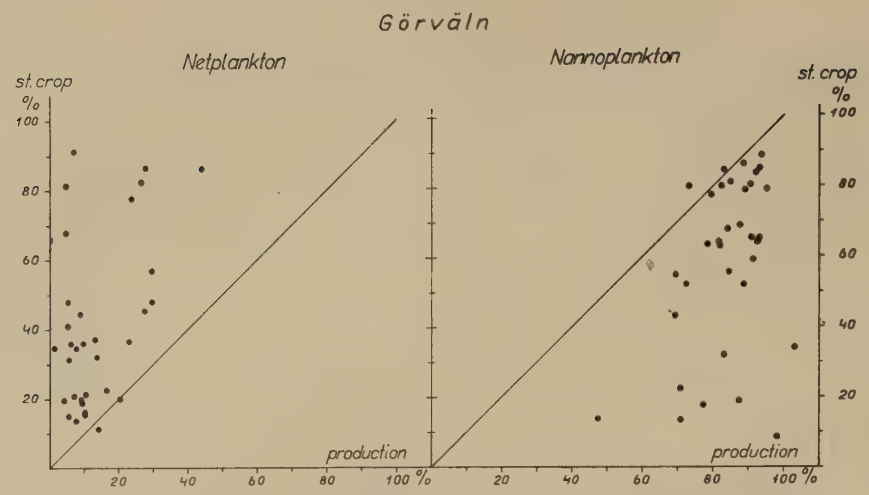

Figure 4. Contribution of netplankters ($\geq$ about 100 μ) and nannoplankters ($\leq$ about 100 μ) to primary production and phytoplankton standing crop in Lake Görväln.

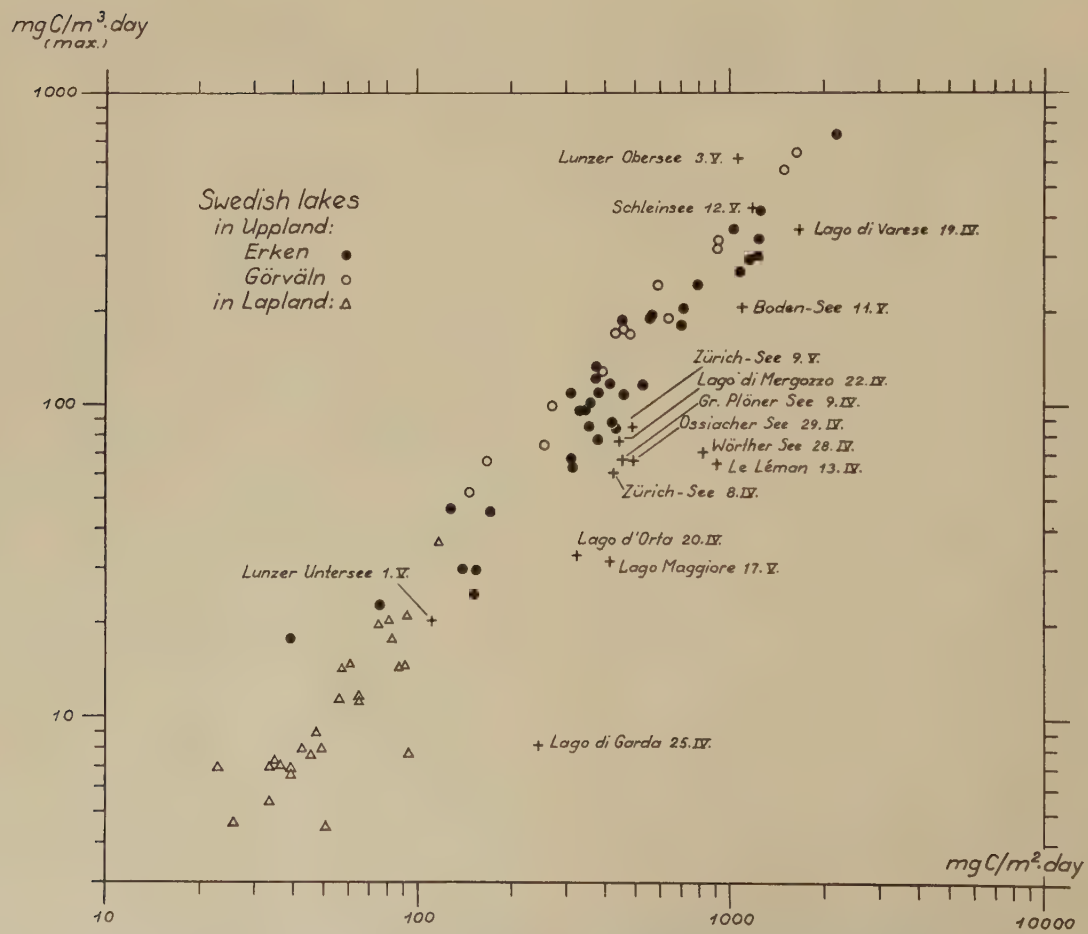

Figure 5. Daily rate of primary production per unit surface (mg. C/m.²) and per unit volume at optimal light (mg. C/m.³, max.) in various lakes according to 24-hours ^{14}C series *in situ*.

how the phytoplankton sometimes is able to continue its growth during several days or even weeks of apparent starvation.

The capability of many algae to store an excess of nutrients when these are easily available should help them to produce organic matter later on, despite low nutrient concentrations in the water. Still more important seems to be, however, that a satisfactory nutrient supply can be maintained also at very low concentrations, if these are compensated by rapid regeneration. According to preliminary results of the author, the daily demand of P and Si for the primary production can amount to or possibly exceed their actual concentrations in the water without affecting them noticeably. At present, we know far too little of nutrient regeneration (mineralization, excretion) to discuss this matter fruitfully. A more dynamic attack, e. g., with use of radioactive tracers (cfr. RIGLER, 1956), seems necessary if the real relations between primary production and nutrient supply are to be elucidated.

Some ^{14}C experiments with additions of small amounts of phosphate, nitrate, and hypolimnic water rich in organic compounds have shown that an increased supply of these substances has a different effect on primary production at different seasons. The effect was greatest during periods of thermal stratification when the impoverishment of the euphotic layer had reached its maximum, smallest during full circulation. A remarkable observation was that the primary production within 24 hours after the addition could be increased as much as 80 % by means of hypolimnic water, whereas P and N never caused an enhancement greater than about 30 %. There are, certainly, many important growth factors besides P and N which urgently call for more detailed studies with the ^{14}C method.

Regional variations

Regional limnology is intimately connected with the trophic concepts, intended to classify lakes according to their main characters. These are determined by numerous morphometric, edaphic, and climatic factors, which all act upon the organic production of the lake. Unfortunately, the terms of oligotrophy and eutrophy are being used in a very variable sense, dependent on whether the factors conditioning the production or the resulting production — or both aspects — are taken as criteria. In my opinion the primary production of the free water, as measured with the ^{14}C method, affords the best quantitative basis for a regional classification of the biological types of lakes (RODHE, 1957). It remains to see, however, if the rate of primary production can be identified with the degree of oligotrophy or eutrophy as usually defined.

In Figure 5, a survey is given for the daily primary production in various lakes investigated by the author and his collaborators. Only ^{14}C series resulting in a curve with a distinct light optimum have been used for this survey (all curves with the maximum at the very surface are omitted), since they alone permit a regional comparison based on (a) the primary production of the whole euphotic layer (mg.C/m².) as well as (b) the primary production in the cubic metre of optimal light (mg.C/m³., max.). In the diagram (Figure 5), the dots for Swedish lakes represent all seasons of the years 1954—1956, except the months with snow-covered ice (some further details in RODHE, 1957), whereas the occasional data from the other lakes were obtained in April and May 1957 (a full report will appear elsewhere).

At first we consider the variations of the production rate per unit surface. The values obtained so far vary from about 20 to 2200 mg.C/m². × day. The mountain lakes in Lappland occupy the range below 100 mg. C/m². × day and the lakes in the fertile district of Uppland (Erken and Görväln) cover the whole upper region of the diagram. These two groups of lakes, those in Lappland and those in Uppland, doubtless belong to different lake types: the former are oligotrophic and the latter eutrophic in all senses of these ambiguous concepts. It might be tempting, therefore, to consider a daily production of about 100 mg.C/m². as a limit between oligotrophy and eutrophy. Apart from the fact that a distinct boundary hardly does exist in nature, the positions of the results from some of the lakes in southern Europe, e. g., Lago Maggiore and Lac Léman (both certainly still regarded as oligotrophic), indicate that such a conclusion would be premature. At present, at least, it seems wise to desist from a connexion of the primary production with the terminology of the trophic system, until more ^{14}C data from various lakes are available.

The temporal variation of primary production, sometimes with great jumps from day to day as described above, makes it impossible to assess a definite place on the abscissa in Figure 5 for a certain lake. Occasional data only permit to state that the production rate in a lake *can* have the value found, they say nothing as to how typical that value is for the lake (the values for Zürich See, e. g., certainly are unusually low because of upwelling hypolimnic water after a northern gale). Nevertheless, these first few results show that several subalpine lakes are able to produce as much organic matter per unit surface as rather productive ("Baltic") lakes in central Sweden. To what extent this productive capacity of great subalpine lakes is original or is due to pollution, remains to be investigated; in any case the very high value of Bodensee—comparable to that of Schleinsee —must be caused by the increased inflow of sewage in recent years.

We then turn to the variations of the production rate per unit volume of the subsurface layer where light is optimal (the ordinate of Figure 5). They range between about 4 and 750 mg.C/m³. × day and the Swedish lakes are distributed, as for the abscissa, over two separate regions of the axis: the Lappland lakes below and the Uppland lakes above 20 mg.C/ m³. × day. As before, the trophic significance of this limit must be left open.

As an interesting difference between the Uppland lakes and the big subalpine lakes we note that the latter produce less organic carbon in the light-optimal cubic metre than the former do at about the same production rate per unit surface. Apparently, regional differences are more conspicuous in the fertility of the water, provided the light intensity is excluded as inhibiting or limiting factor (= optimal light), than they are in the productivity of the water mass as a whole, i. e., per unit surface. This is due to the compensating effect of a thicker euphotic zone when the phytoplankton standing crop is reduced (cfr. Ruttner, 1952, p. 147). To illustrate this with two extreme cases, we compare Lago di Garda with Lago di Varese (Figure 5): per unit volume at optimal light their production rates stand as 1 to 45 (8·1 to 365 mg. $C/m^3 . \times$ day), but per unit surface only as 1 to 6·7 (247 to 1660 mg.$C/m^2 . \times$ day). Another example is afforded by the cluster of eight subalpine lakes in the central part of the diagram (from Lago di Garda to Zürich See, 9.V.): their production differed only within 100 % per unit surface (between 247 and 493 mg.$C/m^2 . \times$ day), but within more than 1000 % per unit volume at optimal light (between 8·1 and 85·5 mg.$C/m^3 \times$ day).

Since the primary production within the subsurface layer of optimal light exhibits such a great regional variation, its determination should be valuable in particular for the biological characterization of different waters. With the reservations given above, whole-day ^{14}C series *in situ* like those used here seem to offer the most reliable way for obtaining comparable data of maximal assimilation per unit volume. It would be more convenient, however, if analogous results could be achieved from experiments *in vitro*, performed at constant light (and temperature) conditions in the laboratory. But such an approach is complicated by the fact that the light optimum of phytoplankton is movable, being higher in spring and summer than in autumn and winter. Thus we cannot expect a simple overall relationship between the assimilation at a given light intensity in the laboratory and that at light optimum *in situ* for all waters and all seasons. Steemann Nielsen (1952, 1954) and Vollenweider (Rodhe, Vollenweider, and Nauwerck, 1957) have derived formulas for the calculation of the primary production *in situ* from experiments *in vitro*, but further work is needed to secure the possible use of routine laboratory ^{14}C experiments for the determination of the light-saturated primary production and its regional variations.

Conclusion

In addition to the aspects of primary production shortly discussed above, the ^{14}C method should permit better insight into the utilization and transfer of energy in the metabolism of fresh as well as marine waters. By means of ^{14}C as tracer the efficiency of primary producers and primary consumers is quantitatively measurable, but for such studies, in particular, it is essential to get full evidence of the physiological significance of the ^{14}C uptake, in the first place its bearing to photosynthesis and respiration (gross and net production).

Limnology and marine biology have so many problems in common when dealing with the productivity of natural waters that a boundary between the two sciences in that field of research looks as artificial as an iron curtain. Every step towards an increased exchange of experience and more active co-operation between limnologists and marine biologists is a progress. Thus it must be greatly appreciated that limnologists have been invited to participate in the symposium on "Measurements of Primary Production in the Sea".

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C. 12.

Diurnal Production and Destruction Rates of Phytoplankton in Lakes

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In the course of the researches on primary production of phytoplankton in northern German lakes two problems of general interest arose: firstly, the fluctuation in the ratio of gross and net production; secondly, the diurnal rhythm of the primary production and the respiration rates. At the same time the findings appear to have a bearing on the various methods of production measurement using water samples suspended *in situ*. Some representative examples have been selected for consideration.

Methods

Measurements were made with the well known light and dark bottle technique, using the Winkler method of oxygen determination. Gross production will be designated as B, the increase of biomass (net community production) as N, and the sum of all destruction processes, including respiration, as R.

In the Ploen Lake (May 14/15) and in the Edeberg Lake (May 23/24) primary production and destruction processes attained the values, given in Table 1, in the bottles of different glass materials, submerged in horizontal position in the lakes for 24 hours (percentages in brackets).

In both series the primary production was from 6·9 to 8·9 % less in the Jena glass bottles, while the destruction rates were the same in each. Therefore net community production was higher in the potassium glass bottles. An investigation of the spectral absorption of these two glasses is contemplated.

Table 2 summarizes experiments with bottles exclusively consisting of potassium glass, suspended in horizontal and vertical positions in the Ploen Lake (May 16/17) and in the Edeberg Lake (May 23/24), (percentages in brackets).

Gross production and destruction were dependent on the position of the bottles. With horizontal exposure, conditions for photosynthesis were more favourable, being 10 % less in the vertical position.

Table 1

mg. C/m.²/24 hrs.

Lake	Potassium glass			Jena glass		
	B	R	N	B	R	N
Ploen	1,120 (100·0)	1,190 (106·2)	—70 (—6·2)	1,020 (100·0)	1,190 (116·7)	—170 (—16·7)
Edeberg	750 (100·0)	980 (130·7)	—230 (—30·7)	700 (100·0)	960 (137·1)	—260 (—37·1)

Table 2

mg. C/m.²/24 hrs.

Lake	Horizontal			Vertical		
	B	R	N	B	R	N
Ploen	730 (100·0)	700 (95·9)	30 (4·1)	640 (100·0)	720 (112·5)	—80 (—12·5)
Edeberg	720 (100·0)	940 (130·5)	—220 (—30·5)	650 (100·0)	980 (150·7)	—330 (—50·7)

Table 3

Daytime	Exposure hours	mg. C/m.² B	R	N	mg. C/m.²/hr. B	R	N
Afternoon	10	310	200	110	31·0	20·0	11·0
		(100·0)	(64·5)	(35·5)	—	—	—
Night	5	—	110	—	—	22·0	—
		—	(35·5)	—	—	—	—
Morning	9	450	210	240	50·0	23·3	26·7
		(100·0)	(46·6)	(53·4)	—	—	—
Whole day	24	760	520	240	31·6	21·7	10·0
		(100·0)	(68·5)	(31·5)	—	—	—

Note: The amount of organic substance mineralized at night was the same as the net community production during the preceding afternoon.

Table 4

Daytime	Exposure hours	mg. C/m.² B	R	N	mg. C/m.²/hr. B	R	N
Night plus day	24	1,900	1,150	750	79·2	48·0	31·2
		(100·0)	(60·6)	(39·4)	—	—	—
Day, evening start	19	1,900	875	1,025	100·0	46·0	54·0
		(100·0)	(46·0)	(54·0)	—	—	—
Day, morning start	19	2,370	836	1,534	124·7	44·0	80·7
		(100·0)	(35·3)	(64·7)	—	—	—

The diurnal course of photosynthesis and respiration

There was evidence of variations of the destruction intensity both between day and night and also at different times during the day. Therefore studies with short overlapping time intervals were made in the Ploen Lake (June 24/25). The results are given in Table 3 (percentages in brackets).

Evidently gross production was much higher in the morning than in the afternoon (50·0:31·0 mg.C/m². × hr.). As the rate of respiration did not change very much (20·0:23·3 mg.C/m². × hr.), the net community increase of organisms in the morning was more than doubled, compared with that of the afternoon (26·7: 11·0 mg.C/m². × hr.); although the exposure of the latter samples started at noon. Doty and Oguri (1957) have suggested an endogenic production rhythm for marine plankton organisms, but this calls for further investigations.

To test our hypothesis that the primary production by phytoplankton[1]) might be suppressed by accumulation of its own chiefly inorganic excreta during daytime, further exposures were made in the Edeberg Lake (May 27/28), in a similar manner as described above but with one set starting in the evening and the other starting in the morning. The results are given in Table 4 (percentages in brackets).

Gross production and net community production in the morning samples were respectively 19·8 and 33·2% higher than in the evening metabolism samples. Obviously, the productivity of the algae had decreased in the bottles at night. Quite another situation was realized in the samples which were taken out of the lake in the morning. Here, in the natural environments, the plankton cells had regained their full production capacity at night. Thus the total recovery of the organisms failed to appear if isolated in bottles.

Importance of the starting time for bioactivity *in vitro*

More detailed investigations with different starting times led to the results illustrated in Figure 1, in which the hourly rates of production and destruction (during the morning, the afternoon, and the night)

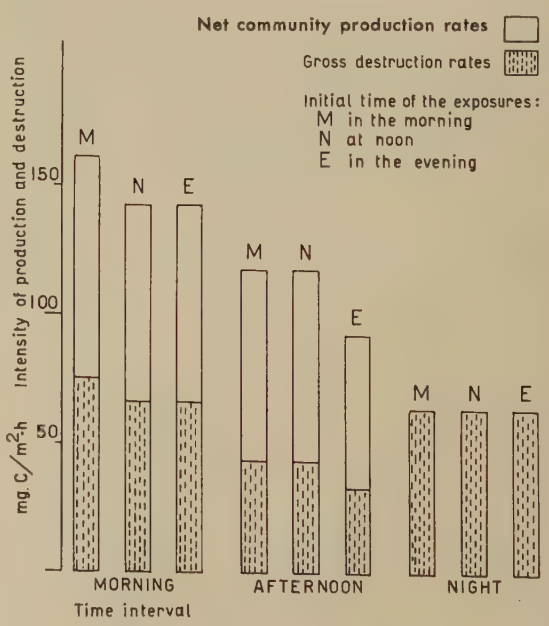

Figure 1.

[1]) *Lyngbia holsatica*, very frequent; *Oscillatoria Redekei* and *Anabaena spiroides*, less frequent. I am indebted to Dr. Utermöhl for the determination of the algae species.

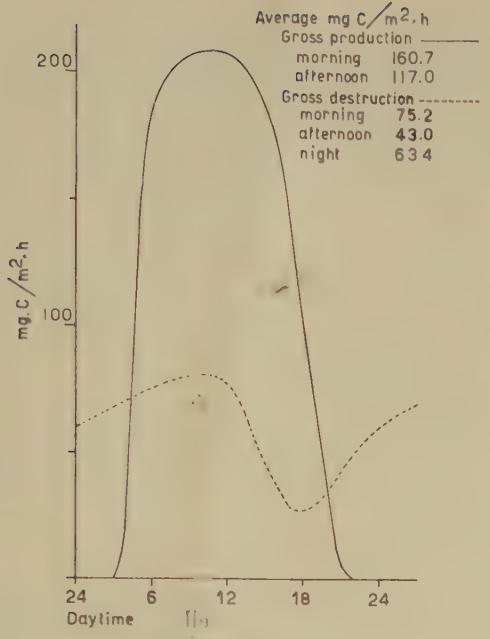

Figure 2.

show a broad shifting of the values depending on the starting times of the exposures. Gross production of the Edeberg Lake was highest for the morning starters (2,617 mg.C/m².24 hrs.) and lowest for the evening starters (2,200 mg.C/m².24 hrs.). The intensities of photosynthesis and respiration changed synchronously in the light and dark bottles, depending on the time of sampling. There seem to exist characteristic states of the organisms and the water, in course of the day. The washing effect of water turbulence is most effective.

It follows from these results that the diurnal course in the rates of production and destruction, for the morning starters, for example, must be of the form illustrated in Figure 2.

Comparison of net community production *in situ* and *in vitro*

It could be shown that the washing effect of turbulence, i. e., the renewal of the contact layer at the surface of plankton cells, takes place faster and more continuously under natural conditions. Therefore the actual production intensities exceed by far those estimated in the bottles at least in highly productive lakes. On the other hand, restriction of primary production caused by the insufficient washing effect also occurs *in situ*, especially in prevailing calm weather.

Oxygen concentrations of the water *in situ* were always measured at the same time as the production. An example is given for the Ploen Lake (July 2/3) in Table 5.

Table 5

Depth, m.	Temp., °C.	mg.C/m.²/24 hrs. *in vitro*	mg.C/m.²/24 hrs. *in situ*[1])
1.....	21·3	90	—56
2.....	20·4	70	84
3.....	19·5	50	240
4.....	19·0	5	195
5.....	18·6	—40	150
6.....	18·1	—55	120
7.....	17·5	—70	90
8.....	16·8	—85	75
9.....	16·1	—100	35
10.....	14·4	—65	2
mg.C/m.²/24 hrs.		—200	+935

[1]) Calculated from the increase of oxygen concentration. The water was supersaturated at a rate of 0·9 p.p.m.O_2 approximately.

In the surface layer of the lake some loss of oxygen to the atmosphere occurred. But oxygen was accumulated in all the other zones of epilimnion. The equivalent quantity of organic carbon must have been formed as new biomass, that is 935 mg.C/m².24 hrs. *in situ*. However, in the suspended bottles any net community production did not develop. On the contrary, 200 mg.C/m². were mineralized out of old stock of organic substances.

Direct measurements in the water may of course, lead to errors, chiefly produced by incontrollable exchange of water masses. Nevertheless the extreme example discussed above clearly establishes the fact that the intensity of the destruction processes *in vitro* considerably exceeds that *in situ*, as a result of differences in the turbulent washing effects.

A comprehensive study of these problems will be published elsewhere.

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D. 1.

Productivity in Relation to Nutrients[1])

By Bostwick H. Ketchum, John H. Ryther, Charles S. Yentsch, *and* Nathaniel Corwin
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Introduction

Brandt (1899, 1902) first suggested that phytoplankton production must be dependent upon the supplies of nitrate and phosphate in natural waters. Proof of this relationship, and of the virtually complete removal of these elements from the water, required more accurate analytical methods than were available at that time. Since the early 1920's however, studies extending from the coast of Norway and the English Channel to the Antipodes have established the interrelationship between these nutrients and the growth of phytoplankton[2]).

The correlation between productivity and nutrients is most clearly demonstrated in moderate to high latitudes where a definite annual sequence of plant production occurs. During the winter season, when phytoplankton growth is at a minimum, vertical mixing, which is greatest at this time, produces a nearly uniform distribution of plant nutrients. As a result of the spring bloom these nutrients are rapidly depleted to low values which persist throughout the summer and much of the autumn. Nathansohn (1906) first pointed out that the stability of the water column, associated with the vernal warming of the surface waters, was intimately correlated with plankton production. Opposing effects of stability are recognized. In a well mixed water column the rate at which nutrients are transferred from the deep richer waters to the surface is maximal, but the plant cells may be carried below the illuminated zone, thus reducing the net production and growth of the population. On the other hand, in a stable water column, the phytoplankton are retained in the illuminated zone, where they can quickly exhaust the nutrients, since the rate of supply is decreased in proportion to the increased stability (Atkins, 1924, 1925; Braarud & Klem, 1931; Braarud, Gaarder, & Grøntved, 1955; Gran & Braarud, 1935; Hart, 1934; Riley, 1942; Sverdrup, 1953, 1955).

Direct estimates of productivity have been made from the removal of nutrients from the water (Atkins, 1923; Cooper, 1938; Kreps & Verjbinskaya, 1930, 1932). The maximum quantity of total phosphorus in the water column in winter has been used as an index of fertility (Russell, 1935; Cooper, 1938; Harvey, 1948, 1950; Armstrong & Harvey, 1950). It should be emphasized, however, that such estimates are minimal unless regeneration *in situ* and the return of nutrients to surface waters by mixing are also evaluated. For example, the data of Redfield, Smith, and Ketchum (1937) indicate that only about 2 % of the total phosphorus cycle between May and November was reflected by changes in the quantity of inorganic phosphorus in the euphotic zone, whereas about 73 % was supplied by vertical mixing and about 25 % by regeneration (Ketchum, 1947). In his excellent evaluation of plant production from changes in phosphate, Steele (1956) shows that only about 18 % of the summer production can be attributed to changes of phosphate in the euphotic zone and 82 % is contributed by vertical mixing. These values do not include the effects of regeneration, which he calculates would increase the original estimate by one quarter to one third. Even in the shallow waters (20 metres) of Long Island Sound, Riley's (1956) estimate of gross production (470 g.C/m^2./year) would require ten

[1]) Contribution No. 946 from the Woods Hole Oceanographic Institution. This work was supported in part by funds from the Atomic Energy Commission under Contract AT (30–1)—1918, and by research grants from the National Science Foundation.

[2]) See, for example: Atkins (1923, 1926, 1926 a); Braarud & Føyn (1931); Cooper (1933); Dankin & Colefax (1935); Gran (1931); Harvey (1926, 1928); Harvey, *et al.* (1935); Hart (1934); Hentschel & Wattenberg (1930); Kalle (1937); Kreps & Verjbinskaya (1930, 1932); Marshall & Orr (1928); Matudaira (1939, 1942); Redfield (1934); Riley, *et al.* (1949).

times the total amount of phosphorus in the water at its maximum concentration (Riley & Conover, 1956). Thus, although the total phosphorus of a water column may be a qualitative index of relative fertility, especially where the water is shallow enough so that the euphotic zone extends nearly to the bottom, the rates of supply of nutrients by mixing and regeneration account for the major part of the nutrient cycle and of plant production.

We need much better information concerning the rate of recycling and reuse of nutrients by the phytoplankton. This is particularly true of oceanic conditions in semi-tropical and tropical waters where repeated observations throughout the year may show little or no change in nutrient concentrations. Plant production in such waters may be entirely dependent upon and limited by the processes of regeneration and vertical diffusion of essential nutrients. In the surface tropical waters of the Atlantic 90 % of the total dissolved phosphorus may be present in organic combination (Ketchum, Corwin, & Keen, 1955). We do not know whether these organic compounds can be used directly by the phytoplankton, nor how rapidly they are decomposed.

Not only is the variation in nutrient concentration in the sea correlated with the growth of phytoplankton, but the various elements change in a related way, and the ratios of change ($\Delta O_2 : \Delta CO_2 : \Delta P : \Delta N$) in the water are similar to the ratios found in the plankton population (Harvey, 1926; Redfield, 1934; Cooper, 1937; Fleming, 1940). These ratios, though less precise than the combining ratios of chemistry, have had a similar stimulating effect on the study of plankton ecology. They have, however, at times been misused, since some have assumed that the ratio of concentration in the sea is similar to the ratio of change, and that the measurement of one element, generally phosphorus since the analytical procedure is the simplest, can be substituted for a more complete study of nutrients. Cooper (1937, 1938) pointed out that the ratio of concentration of nitrate and phosphate departs markedly from a "normal" ratio of 15:1 (by atoms) and that the anomaly is greatest in confined seas, such as the Mediterranean. In the tropical Atlantic we have observed values as great as 60 atoms of nitrogen to one of phosphorus directly below the euphotic zone. Riley and Conover (1956) found an N:P ratio during the winter maxima of about 8:1, about half the value which might be expected for oceanic waters. Furthermore after the spring phytoplankton flowering, the nitrate nitrogen was reduced to negligible quantities, although there was still about 0·5 μg.at./l. of inorganic phosphorus. Our studies in Woods Hole and in coastal waters have yielded similar results, indicating that nitrogen is the serious limiting factor in these waters.

Anomalous N:P ratios may determine the species which dominate a population even when neither element is limiting in the classical Liebig sense. Thus Ryther (1954) showed that an unusually low N:P ratio favoured the growth of *Nannochloris* and of *Stichococcus* which dominated the population of Great South Bay where such N:P ratios were common during the summer.

The foregoing discussion indicates the hopelessness of understanding the mineral nutrition of phytoplankton by determining only one element, unless it has been firmly established that the element is the limiting factor. Even then variations in the ratio may modify the dominance of species. No doubt, determinations of phosphorus and nitrogen, with or without silicate analyses, will also prove inadequate in many cases. Harvey (1947) has shown that manganese may limit growth of cultures. Hutner and Provasoli (1953) and Droop (1955) have demonstrated the need for vitamin B_{12}, and Lucas (1949, 1955) has emphasized the importance of ectocrines, as yet not chemically identified, in controlling plankton production.

Although we have emphasized so far the constancy of the chemical composition of plankton in relation to the essential nutrients, it has been repeatedly demonstrated that cultures of phytoplankton organisms can be grown which accumulate excesses of essential elements or which develop deficiencies which can be so drastic as to completely stop the growth of the population (Ketchum, 1939, 1939a; Ketchum & Redfield, 1949; Rodhe, 1948). Ryther, 1954, has shown that in such nutrient deficient cultures the rate of photosynthesis is reduced until it becomes just sufficient to balance the loss by respiration. In other words, although gross photosynthesis is still carried on by the population there is no net production of new organic matter, a condition which indeed must exist if further growth of the population is stopped by the nutrient deficiency.

There is little or no direct evidence, based upon chemical analyses, that populations in the sea are deficient in composition. Harris and Riley (1956) found that the phytoplankton population in Long Island Sound has a "normal" N:P ratio of about 16·7:1, even though the ratio of concentration in the water was 8:1 or less. However, it is difficult to conceive of the populations which grow in the impoverished Sargasso Sea as being anything other than deficient in these essential elements. We have far too few analyses of the composition of natural populations, and most of these analyses have been made using collections from coastal waters and particularly at times when the population was high, making collecting easy. Dr. John H. Ryther has studied the effects of adding nutrients to surface Caribbean water. Some previously unpublished data, given in Table 1, show that the addition of nitrate and phosphate to natural sea water may considerably increase both gross and net production, but more importantly the addition eliminated the large discrepancy between the ^{14}C and oxygen determination of photosynthesis. This is expressed as the net:gross photosynthesis ratio which is discussed below. In several such experiments addition of phosphate or

Table 1
Effect of enrichment on photosynthesis of natural populations. Surface water exposed for 3 days to natural light in running sea water on deck
(Location 16°40′ N., 65°05′ W.)

Conditions	Type of measurement	Photosynthesis g. C/m³./day	Net: Gross
No enrichment	^{14}C	0·0018	
	O_2	0·0125	0·14
P + N enriched[1]) ...	^{14}C	0·11	
	O_2	0·135	0·82

[1]) 100 μg. at./l. of NO_3^--N, 6·6 μg. at./l. PO_4'''-P.

of nitrate or of both increased photosynthesis, indicating that it was limited by deficiencies of these elements. In some, these additions made no change in low photosynthesis rates, indicating that some other essential nutrient may have been limiting.

In the natural environment and especially in impoverished areas, it is the rate of supply of nutrients through regeneration and physical processes of mixing which can determine how rapidly the population can photosynthesize. When the rate of supply is low the population may always be limited by a deficiency of the element and the net photosynthesis may be always a small fraction of the gross photosynthesis, most of which is required to balance the respiratory needs of the phytoplankton themselves.

This lengthy introduction may imply to some that the relationship between essential nutrients and productivity in the sea is well understood. Such is far from the case. An enumeration of problems may serve to indicate those areas in which additional information is drastically needed.

1. How frequently do the nutrients recycle during the annual production of the phytoplankton population?
2. What is the rate of regeneration of essential nutrients as a result of the decomposition of organic matter? How does this rate vary with temperature, with the concentration of organic matter, with the concentration of bacteria, and with the type of organic matter?
3. What proportion of the phytoplankton production is consumed by the zooplankton population, and how much of the nutrients are excreted by the zooplankton in a form directly usable by the phytoplankton population?
4. What proportion of the organic material produced in the surface waters falls into the deep sea? If, as has been suggested, most of the organic matter decomposes *in situ* and practically all of the remainder decomposes before the organisms and detritus sink to a thousand metres, what is the source of nutrition of the population of organisms living at greater depths?
5. Is the succession of species which dominate populations in the natural environment related to their inorganic nutrition? to temperature? to organic substances promoting or inhibiting growth?
6. How variable is the chemical composition of phytoplankton collected under different conditions in the ocean? Are the phytoplankton which grow in nutrient deficient areas such as the Sargasso Sea comparable with or different from those collected in coastal regions?
7. If nutrient deficient phytoplankton are produced in the sea is their ability to photosynthesize reduced until it barely balances their respiratory needs, as can happen in laboratory cultures?

These questions remain partially or completely unanswered. For many of them we have some estimates, for others there is virtually no answer known.

Investigations in progress

Studies are being conducted in many laboratories throughout the world which may, in time, solve these problems, and answer other questions which are as yet poorly defined. Some preliminary results of an intensive study of coastal plankton populations which is being conducted at the Woods Hole Oceanographic Institution are presented here. This is done, not in the hope of giving any final answers to these questions, but rather for the purpose of outlining the method of attack and of elaborating the questions which we feel are essential to solve.

The observational programme has been planned to include stations in various depths of water ranging from 25 to 1,500 fathoms (see Figure 1). This plan was adopted since the variables which control plankton production may be different in inshore and offshore waters. The programme includes measurements of productivity; of the standing crop of phytoplankton by direct counts and by chlorophyll analyses; of zooplankton by quantitative tows; of nutrient concentrations; of light penetration; and of hydrographic conditions determined by the temperature and salinity of the water. The programme was initiated in September, 1956.

It has become abundantly clear that there is a need for a definition of the physiological state of the phytoplankton organisms in the sea. By microscopic examination one can qualitatively state whether or not cells look healthy, but an independent, quantitative evaluation seems essential before we can fully understand the diverse responses of populations to environmental conditions. Some of our attempts to evaluate various indications of the physiological state of the populations will be discussed.

Net and gross photosynthesis

Photosynthesis measurements have been made by both the carbon-14 and the dark and light bottle oxygen method at all stations on our coastal cruises. A sample of water, obtained in a plastic sampling bottle (VAN DORN, 1956) from a depth of 10 metres,

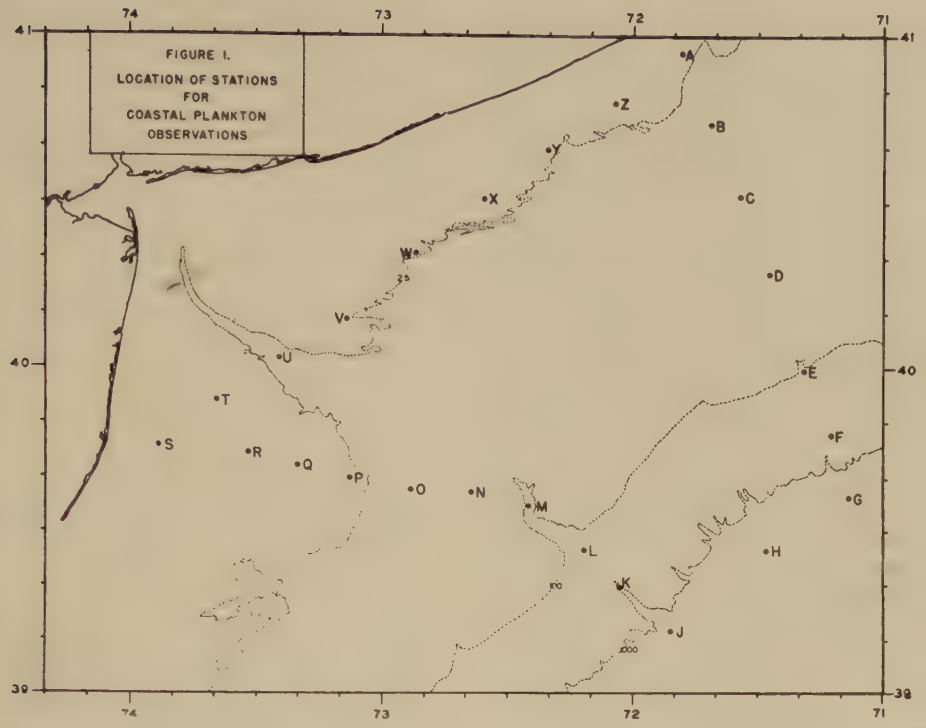

Figure 1. Location of stations for coastal plankton observations.

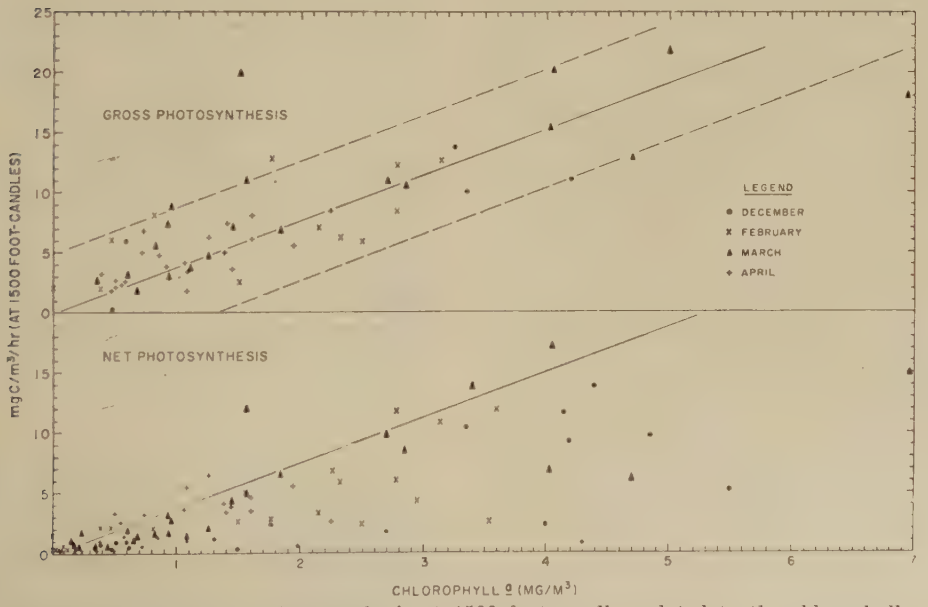

Figure 2. Gross and net photosynthesis at 1500 foot candles related to the chlorophyll *a* concentration. Gross photosynthesis was determined by dark and light oxygen bottle experiments. The envelope corresponds to an oxygen titration difference of ± 0·05 ml/l. Net photosynthesis was determined by carbon-14 assimilation experiments, uncorrected for respiration.

is dispensed into a series of 150 ml. glass stoppered bottles, placed in a tank of flowing sea water in the laboratory on the ship and exposed to an illumination of 1,500 foot candles. The carbon-14 bottles are ordinarily removed after a period of twelve hours whereas the dark and light oxygen bottles remain for a period of 24 hours of illumination. The difference between the oxygen content of the dark and light bottle is taken as gross photosynthesis, and is corrected to the carbon equivalent using an assimilatory quotient (O_2/CO_2) of 1.25. The assimilation of carbon-14, which is not corrected for respiration as suggested by Steemann Nielsen (1952), is taken as the net photosynthesis of the population (cf. Ryther, 1954).

The values thus far observed have been plotted as a function of chlorophyll *a* in Figure 2. Ryther and Yentsch (1957) concluded that gross photosynthesis is closely related to the concentration of chlorophyll *a* and suggest, as an assimilation factor, 3·7 g. of carbon per hour per gramme chlorophyll *a* at optimum light intensity. This correlation line is indicated on the two parts of Figure 2. In general the oxygen measurements scatter at random about this correlation line with considerable individual deviations. Many of the carbon-14 points also approximate this correlation line, but a large proportion are substantially low.

The ratio of net to gross photosynthesis at optimum light intensity may be useful as an indication of the physiological state of the population. This ratio should, in a healthy population, be approximately 0·9 to 0·95 if respiration is 5 to 10 % of total photosynthesis. If, however, the plankton population is not capable of increasing for any reason, the net photosynthesis should be negligibly small and the ratio should approach zero. The mean total gross photosynthesis, and the net photosynthesis, as measured by ^{14}C fixation, for observations at various times of year are shown in Figure 3 (Ryther, unpublished). Five offshore stations—in depths greater than 500 fathoms—and five inshore stations—in depths less

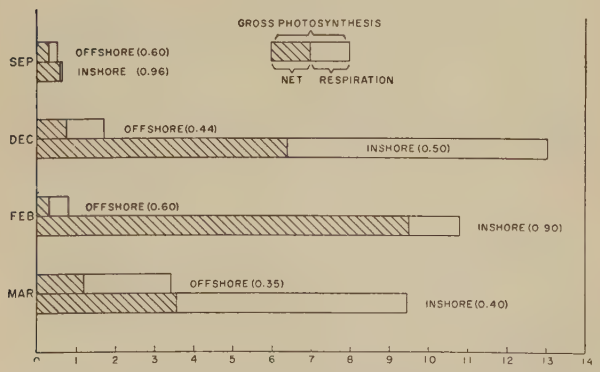

Figure 3. Comparison of net and gross photosynthesis for inshore and offshore (> 500 fm) stations on various surveys. The figures in parentheses are ratios of net: gross photosynthesis.

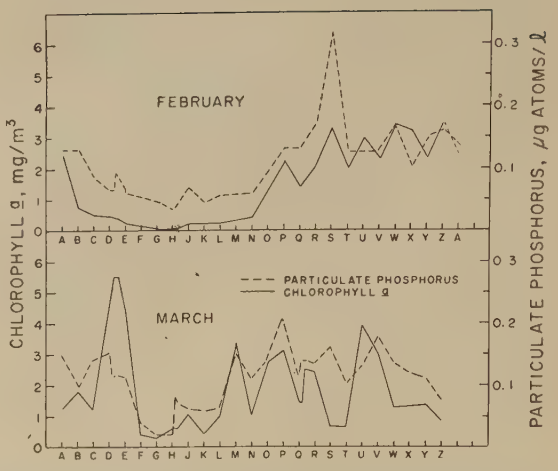
FEBRUARY
MARCH
PARTICULATE PHOSPHORUS
CHLOROPHYLL a
CHLOROPHYLL a, mg/m³
PARTICULATE PHOSPHORUS, µg ATOMS/ℓ
A B C D E F G H J K L M N O P Q R S T U V W X Y Z A
A B C D E F G H J K L M N O P Q R S T U V W X Y Z

Figure 4. Quantities of particulate phosphorus and of chlorophyll *a* in the euphotic zone at the stations shown in Figure 1 during February and March 1957.

than 25 fathoms—were averaged to give these results. Total photosynthesis at the offshore stations ranged from 0·6 to 1·7 mg.C/m³./hr., and the ratio of net to gross photosynthesis ranged from 0·35 to 0·60. At the inshore stations the gross photosynthesis ranged from 0·6 to 13·3 mg.C/m³./hr. The net:gross photosynthesis ratio was 0·9 or more in September and February, indicating healthy growing populations, although the total photosynthesis at these two times was quite different. In December and March, although the rates of total photosynthesis were large, the net:gross photosynthesis ratio was only 0·40 and 0·50.

It seems clear that there are differences in the physiological state of the phytoplankton organisms in the natural environment. We cannot as yet tie these variations in the net:gross photosynthesis ratio with nutrient deficiencies, but this seems to be an almost certain contributing factor especially when one considers that the offshore stations which are typically deficient in nutrients are also typically low in the net:gross photosynthesis ratio. The data in Table 1 indicate that the addition of nutrients to oceanic populations can not only increase the rate of photosynthesis, but can also increase the ratio of net to gross photosynthesis.

Variations in phytoplankton chlorophyll

As mentioned above, our observations suggest that the chlorophyll content is an index of potential gross photosynthesis at optimum light intensity. However, the chlorophyll content of a natural population varies, not only because of changes in the size of the population, but for other reasons as well. Recent observations indicate that the chlorophyll content of populations, both natural and in cultures, undergoes large diurnal variations. Doty and Oguri (1957) described

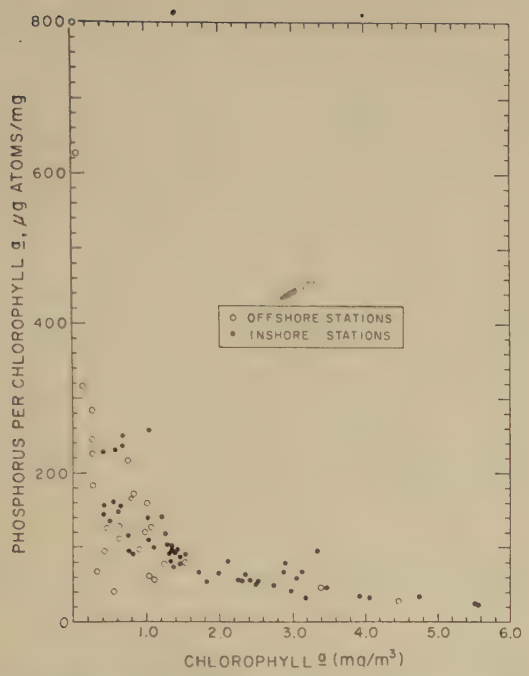

Figure 5. The variation of the ratio of particulate phosphorus per chlorophyll *a* as a function of the chlorophyll *a* content of the water.

a nearly 6-fold variation in the rate of ^{14}C fixation of samples taken from the Pacific depending upon the time of day the sample was collected. YENTSCH and RYTHER (1957) have confirmed the diurnal variation in ^{14}C fixation, though their changes were smaller, and show that the variation is closely associated with similar variations in the chlorophyll content of the population.

The mid-day decrease in chlorophyll is the result of the well-known photooxidation of this pigment (RABINOWITCH, 1945), but changes in chlorophyll content of cells may also be related to nutrient deficiencies. It seems clear, therefore, that chlorophyll alone is an inadequate measure of the standing crop of phytoplankton, though YENTSCH and RYTHER (1957) show that in spite of the diurnal variations it may still be a good index for the maximum gross photosynthesis under optimum light conditions.

Phosphorus : Chlorophyll relationship

It was thought that the ratio of phosphorus to chlorophyll might offer promise of more consistency. Since the nitrogen : phosphorus ratio in these waters is low, phosphorus is probably not directly limiting the population growth. The nitrogen : chlorophyll ratio might, therefore, be more significant, but we have not yet determined particulate nitrogen on large numbers of samples. That the particulate phosphorus and chlorophyll content of the water vary in

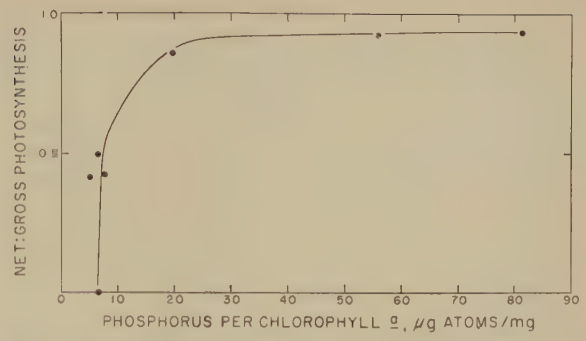

Figure 6. The ratio of net : gross photosynthesis as a function of cellular phosphorus per chlorophyll *a* in a phosphorus deficient culture of *Dunaliella euchlora*.

similar ways is shown by the results of the February and March cruises in Figure 4.

The particulate phosphorus in the water is partly contained in plant cells, partly in organic detritus and partly adsorbed on living and dead organic particles and on silts and clays in suspension in the water. To minimize the physically adsorbed phosphorus our samples are acidified (1 ml. conc. HCl to about 200 ml. of sea water: pH = 1·3) before filtration. A large number of comparisons between acidified and unacidified samples shows that as much as 30 % of the particulate phosphorus may be released by the addition of this amount of acid. Some of the phosphorus released may be extracted from the cell sap of the phytoplankton, but the acidification is not drastic enough to release organically bound phosphorus.

Pursuing the general concept that the chemical composition of plankton organisms is more constant than the environment, one might expect that, in the plant cell, the ratio of phosphorus to chlorophyll would be constant. In cultures of *Phaeodactylum tricornutum* (*Nitzschia closterium* forma *minnutissima*) and of *Dunaliella euchlora* containing adequate phosphorus, this seems to the nearly true, since we have measured ratios ranging from 40—80 µg.at. P/mg. chlorophyll *a*. The relationship observed in natural waters, however, is much more complex, as shown in Figure 5 where the ratio of particulate phosphorus to chlorophyll is compared to the chlorophyll content of the water. At high chlorophyll concentrations the ratio lies between 20 and 40 µg.at. P/mg. Chl. *a*, but at low chlorophyll concentrations the importance of the detrital phosphorus becomes predominant, and the ratio becomes very large. For equal amounts of chlorophyll *a*, the offshore waters, identified by the open circles in Figure 5, appear to contain less particulate phosphorus than the inshore waters. This might be expected since the offshore waters contain lower inorganic nutrient concentrations and less particulate mtater of all kinds. The offshore waters at times, however, have lower chlorophyll concentrations than have ever been observed in the inshore waters. These

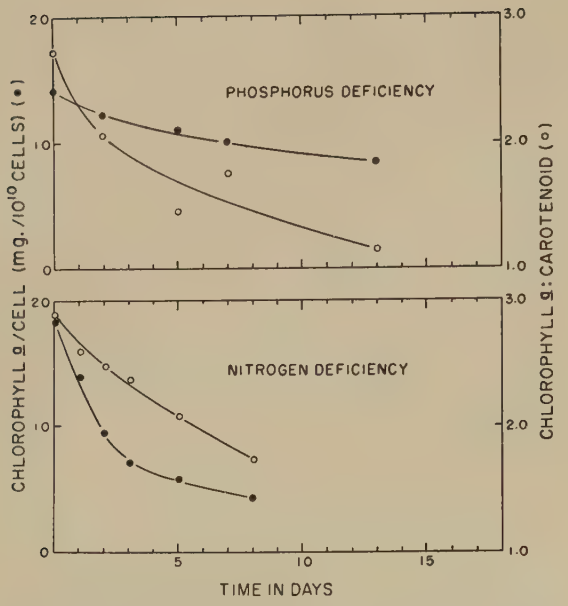

Figure 7. The change in chlorophyll *a* per cell and in the ratio chlorophyll *a*: carotenoid in cultures of *Dunaliella euchlora* deficient in phosphorus or in nitrogen.

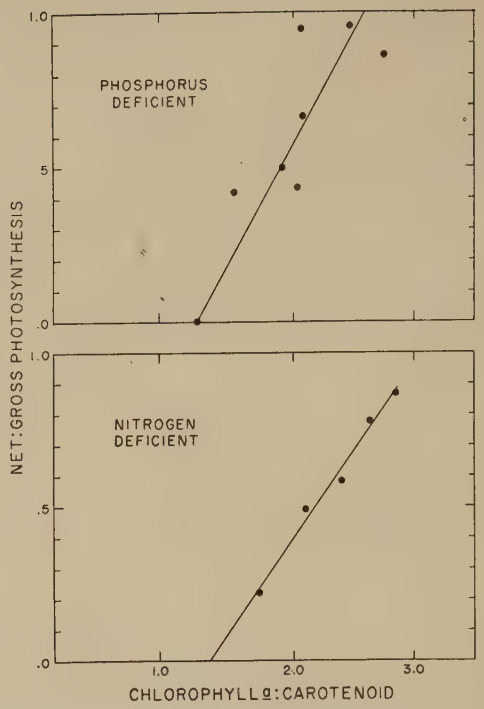

Figure 8. The ratio of net : gross photosynthesis in relation to the chlorophyll *a*: carotenoid ratio in cultures of *Dunaliella euchlora* deficient in phosphorus or in nitrogen.

have the highest ratios of particulate phosphorus to chlorophyll.

The high ratios of phosphorus to chlorophyll observed at low chlorophyll concentrations suggests that the pigment is quickly decomposed as the cells die, but the phosphorus remains bound, for some time afterwards, to the organic detrital material.

In cultures, the development of a deficiency in phosphorus is related to a decrease in the net:gross photosynthesis ratio as is shown in Figure 6, (cf. Ryther, 1954). In this experiment with *Dunaliella euchlora*, this ratio dropped sharply when the phosphorus per chlorophyll *a* in the cells fell below about 25 μg.at./mg. It is interesting and suggestive that this is about the lowest ratio which has been observed in the natural populations. It seems probable that the phosphorus:chlorophyll ratio could be a useful index of the physiological state of natural populations if we could separate detrital from plant phosphorus. Unfortunately we do not yet know how this can be done.

Chlorophyll : Carotenoid relationships

It has long been known that deficiencies, particularly of nitrogen and of iron, lead to chlorotic cells. In a culture chlorosis can be readily detected by eye or expressed as the chlorophyll per cell. In the natural environment, however, very different sizes of cells and the different numbers of chloroplasts in various species make it impossible to state from chlorophyll measurements and cell counts alone whether the population is chlorotic or not.

C. S. Yentsch (unpublished) has observed that the diurnal changes in chlorophyll, described above, are accompanied by comparable, but smaller, changes in the plant carotenoids. The chlorophyll *a*:carotenoid ratio, therefore is more constant than the chlorophyll concentration. However, wide variations in this ratio are observed under different conditions in the sea. In culture experiments, nutrient deficiencies can be shown to produce similar variations. Such a ratio may, therefore, be an index of chlorosis of the natural population.

The decrease of the chlorophyll content of the cells, and of the chlorophyll:carotenoid ratio in cultures of *Dunaliella euchlora* after various times of growth in a medium deficient in phosphorus or nitrogen are shown in Figure 7 (Yentsch, unpublished). The nitrogen deficiency has a greater effect on the chlorophyll per cell but a smaller effect on the chlorophyll:carotenoid ratio than was observed with the phosphorus deficiency. The ratio of pigments may not, therefore, be a precise measure of chlorosis, but a low ratio would suggest a chlorotic, unhealthy population.

In these same experiments net and gross photosynthesis measurements were made from dark and light oxygen bottle experiments at an illumination of 1,500 foot candles. The ratio of net:gross photosynthesis is related to the chlorophyll:carotenoid ratios in these cultures in Figure 8 (Ryther &

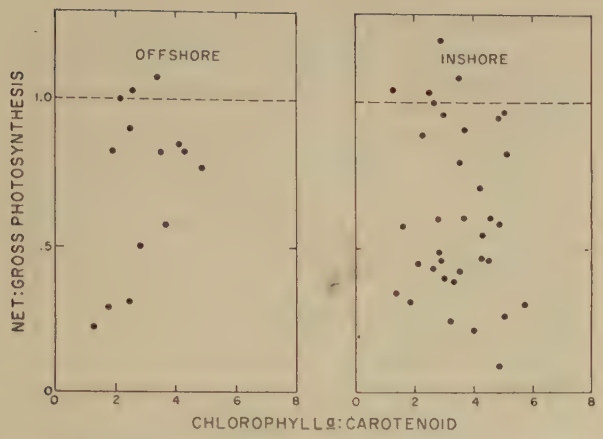

Figure 9. The ratio of net: gross photosynthesis compared to the ratio of chlorophyll *a*: carotenoid in natural populations in the sea.

Yentsch, unpublished). As the nutrient deficiency develops the ratio of net to gross photosynthesis decreases.

The relationship seems much more consistent in the nitrogen experiment, which, perhaps should be expected, since chlorophyll synthesis is more directly associated with nitrogen metabolism. A value of zero for the net:gross ratio would correspond to a nutrient induced compensation point. The results on the cultures indicates that chlorophyll:carotenoid ratios slightly greater than unity correspond to the nutrient deficiency which prevents net photosynthesis. We have observed populations in the sea in which this pigment ratio approaches unity.

Lest we end on too optimistic a note, however, it must be said that the ratio of net:gross photosynthesis is not clearly related to the chlorophyll:carotenoid ratio in the natural environment. These ratios, observed on samples collected at 10 metres of depth at stations during the December, February, March, and April surveys, are compared in Figure 9. In the offshore waters (depths greater than 500 fm.) some indication of a correlation may be present, though the spread of points is much greater than was observed in the culture experiments. In the inshore waters there is no correlation between these two ratios. The population in the offshore waters may be nutrient limited, and this may lead to the fair correlation in this area, but the evidence is certainly unconvincing as yet. No explanation for the numerous low net: gross photosynthesis ratios in the inshore waters which may occur even at high pigment ratios is known.

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D. 2.

Light and the Organic Production in the Sea

By E. Steemann Nielsen

Introduction

Light provides the necessary energy for the plankton algae in transforming inorganic matter such as carbon dioxide, water, nitrate, and phosphate into organic matter. It is, however, only the light absorbed by the pigments active in photosynthesis which is used for this transformation. In the following, photosynthesis will be treated only in outline. It must be stressed that a thorough understanding of the importance of light for the production of organic matter in the sea necessitates familiarity with most of the different aspects of photosynthesis.

The different factors influencing the organic production in the sea—such as nutrients, light, temperature, grazing etc.—can hardly be treated separately. The different factors are generally matched in such a way that they apparently all seem to act as limiting factors at the same time. By varying any of the factors the rate of organic production is temporarily changed. In time, however, a new equilibrium will be established if the new conditions keep constant.

Taking all seas as a whole, it may be stated—as an approximation at least—that the replenishment of the nutrients in the productive surface layers is the essential factor determining the magnitude of organic production. The amounts of nutrients—particularly nitrate and phosphate—which annually are made available for the plankton algae by upwelling, turbulence, and regeneration, determine the total productivity of all oceans.

Generally speaking, it may be stated that light as a whole is available in sufficient quantity to provide the energy necessary for a production equivalent to the annually available P and N in the surface layers. In certain areas or at certain times of the year, light energy is insufficient locally or temporarily. In areas with upwelling, the whole amount of nutrient salts conveyed to the surface is mostly not utilized at that place; the nutrients are distributed over a much wider area by horizontal currents. During winter at high latitudes, light is insufficient for a production utilizing all of the nutrients. Integrated over the whole year and for all seas, light energy is sufficient to keep in step with the replenishment of the nutrient salts. Some areas, for example in the Antarctic, must probably be excluded, because of too strong vertical mixing affecting too poor light conditions for the single algae, leading to a decrease in the organic production. The phytoplankton is not able to utilize the light during the summer to the extent that all phosphate and nitrate near the surface is assimilated (Gran, 1931).

Although light according to the instances presented above cannot be supposed to be the major factor determining the rate of organic production in the sea, it would be incorrect to assume that light is a factor of only minor interest when discussing the background of organic productivity in the sea. First of all at medium and high latitudes, light is a very essential factor. The annual variations in the rate of organic production is largely due to the annual variations in light intensity. The rate of production of organic matter in any area cannot be considered only in terms of an annual total rate. In order to get a real understanding it is necessary to follow the variations from season to season. Here a thorough knowledge of the light conditions in the sea during the year is mandatory.

The dependence on light

When comparing light intensity and rate of photosynthesis several extremely important difficulties turn up. Strictly speaking it is only possible to make such investigations exact if monochromatic light and a homogeneous population of a single plant species is used. The different groups of plankton algae respond in a very different way to light of different wave lengths. Whereas diatoms utilize energy in the blue part of the spectrum up to 50 per cent. better than the energy in the green and the red part, species belonging to the green algae utilize red light far better than blue and particularly green light (cf. for example, Rabinowitch, 1951, Chapter 30).

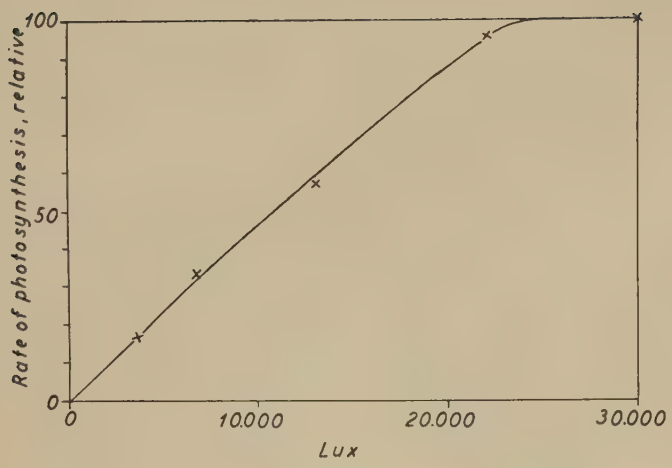

Figure 1. Light intensity (fluorescent light) and rate of photosynthesis in oceanic tropical surface plankton. Maximum rate 0·29 mg.C/m³. hour.

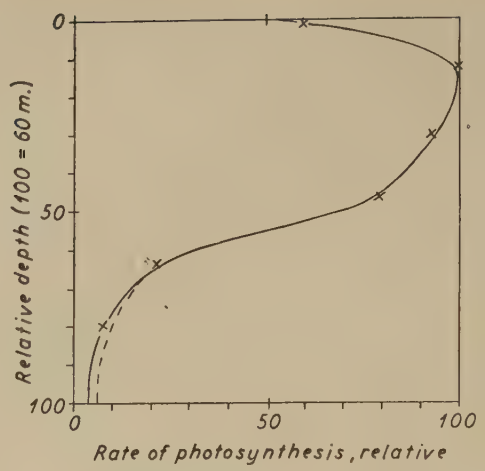

Figure 2. Photosynthesis in water collected at a depth of 10 m. when exposed at different depths. Entrance to the Gulf of Siam.

However, it is of course possible to make a light-assimilation curve showing the dependence of rate of photosynthesis on light intensity—using any light source and any mixture of species. By varying the light intensity it is, however, mandatory that the proportion between the energy of the different wave lengths keeps constant at all light intensities. If the light intensity when using sunlight is varied by lowering bottles with the plankton into different depths of the sea, in most cases the quality of light will vary considerably from one depth to another. A correct light-assimilation curve is therefore by no means obtained by measuring the light energy at the different depths and plotting the rate of photosynthesis against the measured light intensity.

In laboratory experiments neutral glasses are often used for varying light intensity. Very often these neutral glasses are by no means strictly neutral. First of all in the transition area between red and infra-red the transparency may vary. Especially if incandescent light is used which has the maximum energy just around these wave lengths, important errors may turn up. Screens consisting of one or more layers of a wire sieve are mostly better than neutral glass for such a work. In this case it is, however, necessary that the area of the light source is great—for instance consisting of some rows of smaller bulbs—and that the experimental bottles are placed on a rotating wheel.

If monochromatic light is used, the light energy is given in calories per time and area or in any similar way. If white light is used, light energy is mostly given in units of lux or foot candles. Such units have, however, only a definite meaning when measuring light in connexion with the human eye. As the different plant species have very varying spectral sensitivities it is impossible to construct any units of "photosynthetic lux" with general application. Using light sources of different spectral composition it is, however, meaningless to compare the photosynthetic responses by giving the light energy in calories.

In photosynthetic work using non-monochromatic light all light measurements, in whatever units they are presented, may only be compared *inter se* if the same light source has been employed. In this case, however, very similar light-assimilation curves are obtained from plankton from the same sort of water. During the "Galathea" expedition in tropical-subtropical water 12 of such curves were obtained in fluorescent light (see Steemann Nielsen and Aabye Jensen, 1957) and in 1956 and 1957 about 100 such curves were obtained during cruises of the "Dana" in the North Atlantic and in arctic waters using incandescent light. Figure 1 presents an example of such curves.

Light of a high intensity has an inhibiting effect on the photosynthesis of plankton algae. A discussion of the physiological background of the inhibition is given by Steemann Nielsen, 1949 and 1952. It must be considered of a rather complicated nature. The light inhibition directly observed at very high light intensities seems first of all due to a chlorophyll-activated photo-oxidation decomposing enzymes of the photosynthetic mechanism. A chlorophyll inactivation of some kind seems, however, mostly to be found at the same time. This inactivation represents a defence at high light intensities against a too pronounced chlorophyll-activated decomposition by photo-oxidation of cell constituents. Ryther (1956) has presented a series of light-assimilation curves within the entire range of light to which phytoplankton may be exposed in nature.

In the tropics and subtropics and during summer in temperate waters a pronounced light inhibition is always found near the surface in bright weather (see Figure 2). As it takes a considerable time for

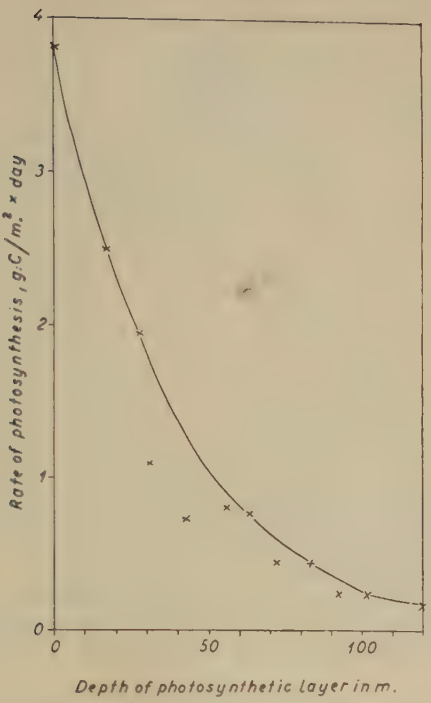

Figure 3. Depth of photic layer and maximum rate of photosynthesis per m². surface.

the reactivation of the photosynthetic mechanism, it is important to keep the duration rather long (4 hours) of experiments with surface water at a definite light intensity (tank experiments). It is especially important, if the water is collected late in the afternoon. Talling (1955) and Edmondson (1956) have given useful information regarding the relation of photosynthesis by phytoplankton to light in lakes. An early important marine contribution was that of Jenkin, 1937.

The utilization by the algae of the light penetrating the sea surface

Compared with the light energy penetrating the surface of the sea only a small amount is utilized by the algae. According to Kimbal (1928) the average daily light energy at a sea level during a year may be put at about 300 g. calories per cm². in the tropics and subtropics. Half of the energy is in the visible part of the spectrum. This part is practically identical with that used in photosynthesis. The average daily production of organic matter in the sea may be put at $1{\cdot}5 \times 10^{-5}$ g. carbon per cm². (see Steemann Nielsen, 1954), corresponding to 0·06 g. calories per cm². This is a utilization of only 0·02 % of the total energy and about 0·04 % of the energy in the visible part of the spectrum.

When growing under light of very low intensity, both algae and terrestrial higher plants may utilize as much as 20 % of the incident energy in the visible part of the spectrum under optimal conditions. When they are growing in full daylight, the maximum conversion is reduced to about 2 % of the visible part of the spectrum (Burlew, 1953, Chapters 1 and 5). In the sea a conversion of nearly the same size may occasionally be found—Walvis Bay (Steemann Nielsen, 1954).

The reason why the marine plankton algae normally utilize only a small fraction of the light energy falling on the surface of the sea is, according to all evidence, not due to a poor utilization of the light energy absorbed by the pigments in the algae. The assimilation number, i. e., mg.CO_2 assimilated per mg. chlorophyll per hour, seems always to be relatively high.

The reason why only a minimal percentage of the incident light normally is utilized by the algae in the oceans is to be found predominantly in the fact that only a minimal percentage of the light is absorbed by the pigments of the plankton algae. The rest is absorbed mainly by the water but also to some degree by particulate dead material and by coloured dissolved organic matter, e. g., "yellow substance".

A high utilization of the incident light is possible only if the plankton algae are concentrated in a shallow photosynthetic layer. The light absorption by the water is thus minimized. In the basins of the pilot plants used for growing algae, a daily yield (net production) of up to 6 g.C per m². surface has been obtained (Burlew, 1953). The corresponding gross production is about 8 g.C. The depth of the water is only a few cm. in these basins. The depth of the photosynthetic layer was only 0·8 m. in Walvis Bay where the highest rate of photosynthesis was measured during the "Galathea" expedition—3·8 g.C per m². per day.

In a theoretical ocean of absolutely pure water 1 % of blue light (475 mμ) would, according to Dr. Jerlov, Gothenburg (personal communication) be found at about 160—165 m. and 1 % of green light (525 mμ) at about 90—95 m. The depth of the "photic layer" of this plankton-free and production-free "ocean" would be about 140 m. deep. This is not particularly much deeper than found in the most transparent parts of the oceans such as the Sargasso Sea. Therefore only an inconsiderable standing crop of plankton can be found here.

The rate of organic productivity per m². is—as to be expected theoretically—inversely proportional to the depth of the photic layer. Figure 3 illustrates this clearly. The curve shows the maximum rate of production found at every thickness of the photic layer according to the measurements on the "Galathea" expedition. The stations were divided into 10 m. classes according to the depth of the photic layer.

If the rate of production per m². is definitely lower than the curve values, the opacity of the water must be due primarily to causes other than living plankton algae. It must be mentioned here *inter alia* clay — brought out by rivers and remnants from a consider-

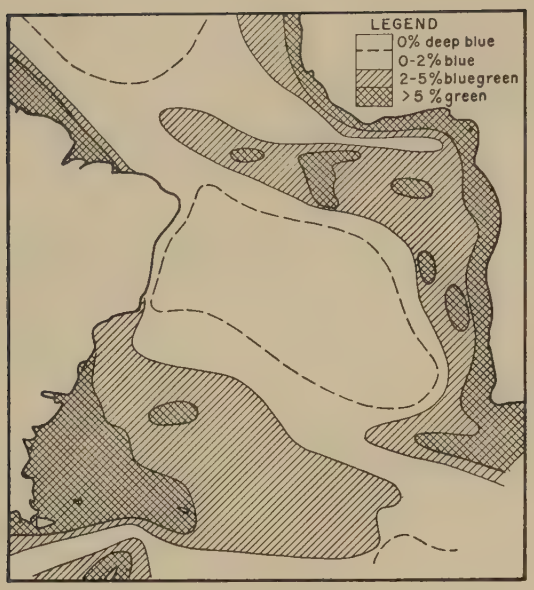

Figure 4 a. Distribution of colour of the sea. (After Schott.)

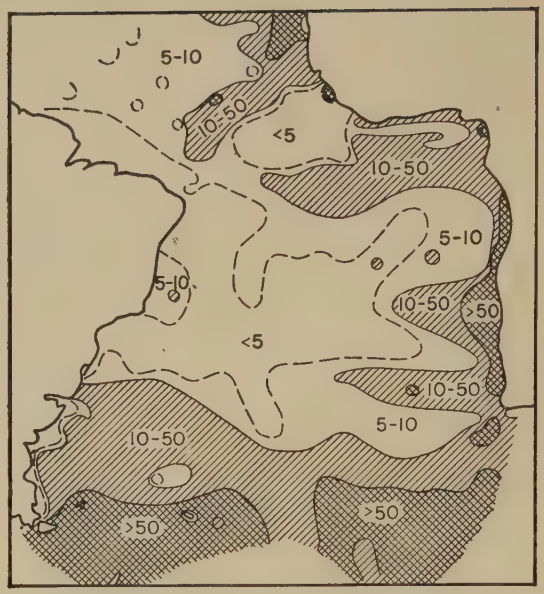

Figure 4 b. Distribution of plankton, numbers in thousands per litre (average 0–50m.) South Atlantic after Hentschel 1933.

able production which has been found previously in the water mass — "yellow matter", and detritus. The last mentioned influence may be found at the same place where the high productivity has taken place—such as found after a spring maximum at higher latitudes—or the influence may be found at another place due to horizontal transport of the water. A typical example of the latter was found by "Galathea" in the area of the Atlantic immediately north of the Benguela Current proper.

The lesser the influence from land the more the transparency of the water is influenced by the algae alone. In freshwater lakes the influence from land is especially conspicuous. Whereas, according to the "Galathea" stations, in the ocean a gross production was found of up to nearly 2 g. C per m^2. surface at a depth of the photic layer of nearly 20 m. (Benguela Current); in Japanese lakes (Ichimura, 1956b) at a transparency of 5 m. (depth of the photic layer 11 m.) the gross production during summer in maximum was 1·7 g. $C_6H_{10}O_5/m^2$./day (= about 0·5 g. C).

The water colour is dependent on the transparency of the water. Therefore, the distribution of the water colour in the open ocean outside the influence of land must be closely similar to the quantitative distribution of plankton algae. The charts from the South Atlantic presented in Figure 4 after Hentschel (1933) show this to be true.

In discussions on light utilization, the pigments in the algae—first of all chlorophyll—may be considered especially suitable for estimating the standing crop of algae. An important question to be answered is how much chlorophyll (+ other pigments) may at maximum be found in the photic layer. In order to get as high values of the pigments as possible the depth of the photic layer must be as small as possible. Otherwise the light absorption by the water itself will be too important. The most ideal conditions should thus be found in the basins of the pilot plants were *Chlorella* and other algae are cultivated. In one of the most successful experiments from USA, presented by Burlew (1953, p. 251), the standing crop of algae was held constant at a value equal to about 300 mg. chlorophyll per m^2. surface. The depth was 7 cm. The concentration of chlorophyll was computed from the values of dry weight by using Kok's conversion factor for *Chlorella* grown outdoors—presented in the same paper, p. 65. The same chlorophyll concentration per m^2. was found in similar German experiments (see Table 2 by Burlew, 1953, p. 175).

As shown by Gessner (1949) the chlorophyll concentration per m^2. surface may be considerably larger in shallow, very eutrophic freshwater lakes. It must, however, be stated that by far most of the chlorophyll —and the algae—in such localities is found below the compensation depth. According to the table presented by Gessner, p. 383, 606 mg. chlorophyll was thus found per m^2. in the "Wessling See" on 5. August. The depth of the photic layer was, according to the vertical distribution of oxygen, about 2·5 m. 148 mg. chlorophyll m^2. was found in these 2·5 upper metres.

Figure 5 presents the dependence of chlorophyll/m^2. in the photic layer on the depth of the photic layer in Japanese lakes. The curve is drawn on the basis of Figure 2 by Ichimura (1956). The values for the depth of the photic layer are converted from the values for transparency actually presented by using the con-

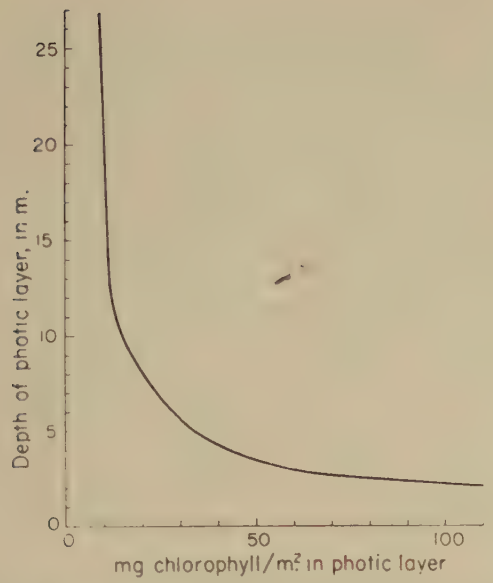

Figure 5. Chlorophyll/m². in the photic layer as a function of the depth of the photic layer. Japanese lakes according to Ichimura 1956.

version factor 2·2 presented in Table 1 by Ichimura (1956 b). The values computed according to the actual single determinations by Ichimura show without exceptions a chlorophyll concentration per m². surface in the photic layer definitely below 200 mg. The value 300 mg. chlorophyll/m². in the photic layer according to the *Chlorella* pilot plant experiments may thus really be considered as maximal.

In terrestrial plant communities a chlorophyll concentration considerably higher than 300 mg./m². is often found. All the chlorophyll is here found in the zone where normally a positive production of organic matter takes place. According to Gessner (1949) thus 1000 and 1300 mg. chlorophyll per m². was found in a meadow and a beach forest, respectively.

It seems thus that a concentration of chlorophyll nearly 5 times as high is possible in a terrestrial community compared with that in a planktonic. At first sight this may perhaps seem astonishing. It is, however, easy to understand if we consider the light perception of the individual chloroplasts in plankton algae and in ordinary terrestrial plants, respectively.

In plankton algae any single chloroplast is exposed to the maximum light intensity found in a plane normal to the direction of the light. Near the surface in bright light the chloroplasts are therefore exposed to supra-optimal intensities giving rise to light inhibition. Theoretically, it is possible to imagine that the light energy penetrating a definite surface of the sea or a lake was divided into, for example, 10 equally large parts each penetrating a surface equal to the original surface. No light inhibition would now be found. According to Figure 6, to be discussed later, the

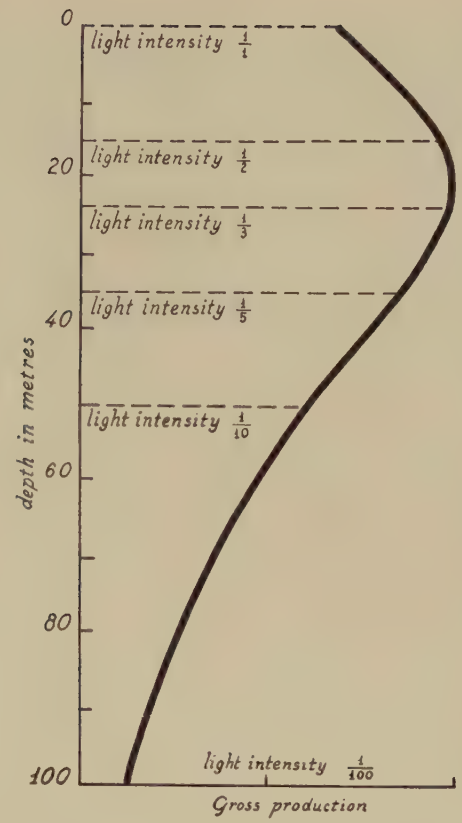

Figure 6. Light intensity and rate of gross production at the different depths. Cf. the text.

total rate of photosynthesis for all 10 surface units would be about 2·5 times greater than the rate found when all the light energy penetrated a single of the surface units. The amount of algae and chlorophyll in the photic zone of the 10 surface units would be about 5 times the amount below the original single surface unit—see Figure 6.

In a community of terrestrial plants, nature has created an architecture and a placing of the leaves such as to imitate the conditions in the theoretical plankton example where the light energy penetrating a surface unit theoretically was divided into several equal parts each penetrating a separate surface unit. This is done in several ways.

Firstly, the leaf area (one-sided) is several times greater than the area of the soil—in a beach forest about three times. Boysen Jensen (1932) has instructively called attention to the fact that in most communities of terrestrial plants the leaves are placed in such a way that the light utilization at high light intensities is markedly increased compared with the utilization possible if the area of the community was covered by a single horizontal layer of leaves. The large area of the leaves is especially of importance in a forest because the angle of incidence of the light

constantly vary, even in a mild wind due to the flexibility of the leaves and the branches. Secondly, the epidermis of the leaves—first of all in sun leaves—functions as a screen which rather effectively diffuses the light. The upper parts of the chloroplasts are situated close up to the cell walls of the palisade cells and the latter are surrounded by intercellular spaces which effect a rather total reflection of light on the passage from cell to air. The palisade cells thus function as more or less individual light channels on the side of which the chloroplasts, because of the diffusing effect of the epidermis, never receive the full strength of the light. The light energy penetrating the surface of the leaf is thus divided over a much larger "inner surface". Both by enlarging the real surface area of the leaves and by enlarging the "inner surfaces" of the leaves a condition is created very similar to the theoretical plankton experiment presented above. The light conditions inside leaves were discussed by Steemann Nielsen (1940). Model experiments were made at the same time. By using as an epidermis an opal which diffused the light to the same degree as an ordinary epidermis of a sun leaf, the light energy which was received at the wall of a "model palisade cell" was reduced in maximum 1/5. One result of the instances mentioned is the lack of light inhibition in sun leaves. It is thus clear why the amounts of chlorophyll per surface area in terrestrial communities are up to 5 times greater than those found in maximum in the photic layer of a plankton community.

The energy from the sunlight which on the whole is utilized in photosynthesis may roughly be divided into three equally large parts, a blue, a green, and a red part. In terrestrial plant communities the red part is, if anything, the most important one. In transparent oceanic water red light on bright days is, on the other hand, without any importance at all, or better: the production per surface area would increase if the red light from the sun was filtered off. This paradox is based on the very high absorption rate by the water in the red part of the spectrum. Already at a depth of 15 m. the red light is reduced by the water to such an extent that it is without practical importance for photosynthesis. Integrated for a whole day light inhibition on bright days in the tropics and subtropics is found down to a depth of about 20 m. if the depth of the photic layer is about 100 m. (see Figure 6). By removing the red part of the light, light inhibition would be reduced near the surface whereas the photosynthetic rate below the zone of light inhibition would be unaffected. The joint effect would thus be an increase in production.

The influence of weather conditions on the rate of organic productivity

When discussing the influence of weather conditions on one hand, we must distinguish between areas with more or less static conditions such as the major tropical-subtropical parts of the oceans and, on the other hand, areas at higher latitudes.

In the latter case the influence of weather conditions may be of a rather complicated and intricate nature. One of the main influences of weather here concerns stabilization of the water masses. In many areas this is the very factor determining the onset of phytoplankton production in spring. This will be discussed below. Another similar influence of weather conditions is found for example during summer in the northern North Sea where Steele (1956) has shown the importance of gales for the vertical mixing and thus for the replenishment of nutrients in the photic layer.

Here we will only discuss the direct influence of the variations in light due to weather conditions. The variations from day to day in the light energy penetrating the surface is especially important when the maximum energies are small such as during winter in temperate waters. Phytoplankton production is possible even during the darkest time of the year in the transition waters between the North Sea and the Baltic because of the salinity-conditioned strong stabilization of the water masses—cf. this volume, p. 102. In bright weather in the middle of the day during the darkest time of the year the light intensity is just high enough to effect optimum rate of photosynthesis just below the surface. Therefore any reduction of the light intensity due to clouds will seriously affect the rate of production. During a real dark day the light intensity is often reduced by 90 %. No positive production is found during such a mid-winter day.

In the subtropics and tropics but also during summer at higher latitudes the influence of the light variations from day to day is much less. Let us consider a tropical station at which the depth of the photosynthetic layer is 100 m. If the plankton algae are evenly distributed in the layer—and the light absorption is the same at all depths—we obtain the hypothetical curve shown in Figure 6. This represents the daily relative rates of total photosynthesis at the different depths on a clear day. The maximum rate is found at depth of about 20 m., where the average of the blue and green parts of the light falling on the surface is reduced to nearly one-third. As the rays of the red part of the spectrum are completely absorbed in the upper 5 to 10 m., for the sake of clearness, although not correctly, we may disregard this part of the light. The light intensity being too high near the surface causes a reduction in the rate of photosynthesis.

If the light falling on the water surface is reduced throughout the day to one-third, the rate of photosynthesis at the surface is the same as that taking place at a depth of 24 m. on a clear day. With a decrease of the light intensity to one-third, the photosynthetic rate per unit volume of surface water (g. C/m^3.) thus increases by nearly 50 %. However, the photosynthetic layer is now only 76 m. deep. The curve for a light intensity of one-third is identical with that (Figure 6 light intensity 1/1 at the surface) which would be obtained if the curve is shifted bodily 24 m. upwards. The rate of total photosynthesis

measured per square metre (g. C/m².day) is thus about two-thirds of that measured at the light intensity 1/1 at the surface. By reducing the light intensity at the surface to one-tenth, the curve (Figure 6) has to be moved bodily 50 m. upwards, which reduces the depth of the photosynthetic layer to 50 m. The rate of total photosynthesis at the surface as g.C per cubic metre per day decreases about 15 % (or less—because of the red light) and the rate as g.C per square metre per day decreases to about 25 % of the rate at light intensity 1/1 at the surface[1]).

A day with a light intensity of only one-tenth of that on a normally clear day is in fact a very dark one, for example one on which rain falls throughout the day. Only in such very dark weather does a slight reduction of the photosynthetic rate occur (per unit volume) at the surface.

The rate of photosynthesis below a unit area is affected somewhat more by weather conditions, but the rate is seldom reduced by more than 50 %. On extremely dark days only the rate is reduced by about 75 %.

Although a reduction of the light penetrating the surface will reduce momentarily the organic productivity per area in all cases, this does not mean that such variations are of importance for the production over a longer period. A factor like light may at a given moment act as limiting. Integrated for a longer period it is, however, mostly another factor—replenishment of nutrients—which is the real limiting factor for organic productivity.

The influence of vertical stabilization of the water masses

A plankton alga has to follow the water masses carrying it. If the latter are vertically instable the algae may, therefore, make extensive vertical movements. In order to be able to grow, algae must stay during a sufficiently long time of the day in the illuminated upper layers. Very often this is not the case in waters without an effective stabilization of the surface layer. Braarud and Klem (1931) were the first to call attention to the importance of instability. Their views have been corroborated in a number of successive investigations in northern waters and in the Antarctic.

Recently, Sverdrup (1953) has theoretically tackled the problem in an attempt to explain the inset of the vernal blooming of phytoplankton in the Norwegian Sea at Weather Ship "M". According to Sverdrup there must exist, for each time of the year, a critical depth such that blooming can occur only if the depth of the mixed layer is less than the critical depth. With certain assumptions it was possible to compute the critical depth. At Weather Ship "M" on 7. April the thickness of the mixed layer was for the first time less than the critical depth, but a shallow mixed layer (stabilization) did not appear before mid-May. In accordance with these conditions a moderate blooming started at the end of the first week of April followed by an intense blooming in the second week of May.

In the northern waters stabilization seems generally to start so early in the year that practically all amounts of the nutrient salts found above the instability layer are utilized by the algae during summer. In many parts of the Antarctic where an effective stabilization is often found for a relatively short time of the summer the nutrient salts are found in much higher concentrations at the start of the stabilization period compared with northern waters. The algal bloom does not manage to use all of the nutrients available. Here light may thus be assumed to be the real limiting factor for the annual organic production by the plankton, a situation only very rarely met with in the sea.

[1]) In his experimental work using plankton cultures exposed in a lake, Talling (1955) has corroborated the theoretical considerations presented above (see his Figure 5).

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D. 3.

The Effect of Grazing in Reducing the Primary Production: a Review

By D. H. Cushing

The effect of grazing may be determined by the analysis of phytoplankton populations at sea or experimentally in a bottle. The experimental method is really the same as that used in the analyses of populations at sea, but a little simpler. In this review, the two methods will be treated separately and it will be shown that the measure of grazing obtained from the experimental work is not necessarily applicable to the populations at sea.

Experimental Work

The instantaneous coefficient of grazing mortality, G, in an algal population which is not reproducing may be defined:—

$$G = -\frac{1}{(t_1 - t_0)} \cdot \log_e \cdot \frac{P_1}{P_0}$$

where P_0 is the number of algae per unit volume at time t_0, P_1 is the number at time t_1. Many workers express their results as "volume swept clear", F. Let V be the volume of culture medium per animal, then $F = VGt$, where t is unit time. From estimates of algal density, the rate of food intake per animal/day may be found, i. e., FP/day.

Experiments are usually carried out for about 24 hours in small vessels which may or may not be agitated. Light and reproductive rates have not been measured, although the latter have sometimes been minimized, with the use of control bottles without grazers.

Volumes swept clear, as measured by various authors, are set out in Table 1. A few trends are discernible:—

1. There is basic filtering rate, independent of selection, by which particles as small as carmine are taken up. As these (and μ flagellates) escape the filtering setae of *Calanus* and *Acartia* (Conover, 1956) the measurements of their uptake represents a minimum of "blind filtration" and cannot be used for estimates of food requirement.
2. Harvey (1938) has shown that *Calanus* V will select *Lauderia* in preference to *Chaetoceras*. Marshall and Orr (1955) have shown that *Calanus* ♀ took *Prorocentrum micans* in preference to *Peridinium trochoideum*. Hence the food must be taken in partly by some other way than blind filtration. As feeding continued in full darkness (Gauld, 1953), it is likely that the selection takes place on encounter by means of the many hairs, aesthetes, etc. which are carried on the antennules of copepods.
3. Fuller (1937) showed that F varied with temperature for *Calanus* and Conover (1956) has shown that for *Acartia*, F increases with temperature. This is probably a relation between temperature and activity.
4. Gauld's work (1951) shows that for the same concentrations of *Chlamydomonas*, F increases as the size of the grazer increases, ($F \propto L^2$).
5. Harvey's (1938) results show a clear relation between F and the size of the food organism; the same result emerges from Marshall and Orr's (1955) work, but less clearly.
6. Volumes swept clear range from 1—240 ml./day for *Calanus* V and ♀, those of Marshall and Orr (1—40) being on the whole less than those of Gauld (4—70) and Harvey (5—240). It is possible that the size of the experimental vessel plays a part here, because Harvey used $^1/_2$ litre beakers, which are much larger than any vessel used by any other workers. This possibility has been confirmed recently in a grazing experiment carried out by myself and H. F. Nicholson. Culture bottles of 5 ml., 25 ml., 100 ml., and 500 ml. were used; two specimens of *Temora longicornis* were put in each in a culture of *Skeletonema costatum*. The experiment was run for 24 hours under constant light intensity at constant temperature with slight agitation; the degree of

Table 1. Estimates of "volume swept clear"

Animal	Food	Size μ	Density	F	Author
Calanus V	Carmine	—	—	5·6	Fuller and Clarke, 1936
Calanus V	*N. closterium*	—	—	0·35 (3° C.) 2·83 (8° C.) 1·09 (13° C.)	Fuller
Calanus	*N. closterium*	4	154,500/ml.	5	Harvey
	L. borealis	30	1765—220/ml.	66	
	D. Brightwellii	40	79/ml.	240	
	Chaetoceros sp.	?	?	—	
Pseudocalanus minutus	*Chlamydomonas*	3	10/100/μl.	4·28	Gauld
Temora longicornis	,,	,,	,,	8·38	
Centropages hamatus	,,	,,	,,	12·99	
Calanus V	,,	,,	,,	64·36	
V	,,	,,	,,	71·03	
IV	,,	,,	,,	36·65	
III	,,	,,	,,	22·24	
Acartia clausi	*S. costatum*	8×3		5·25	Conover
Acartia tonsa					
Calanus ♀	*Skeletonema costatum*	4	11—228/μl.	4·25	Marshall and Orr
	Lauderia borealis	30	6—1590/ml.	4—32·9	
	Skeletonema costatum	4	0·77—288/mm^3.	1·3—23·9	
	Chaetoceras decipiens	50	1—995/ml.	1·1—22·4	
	Ditylum Brightwellii	40	1—63/ml.	7—11·4	
	Chlamydomonas pulsatilla	19×12	620—6200/ml.	3·21—11·83	
	Platymonas carteriaformis	15×23	415—41,500/ml.	1·4—5·15	
	Chromulina pusilla	1—3	16,360—1,636,000/ml.	0·44—2·24	
	Monochrysis lutheri	6×6×2	1880—188,000/ml.	0·22—0·45	
	Dicrateria inornata	3—5·5	10—2180/mm^3.	0·16—0·84	
	Prymnesium parvum	10×5	0·4—40/mm^3.	0·37—1·77	
	Syracosphaera elongata	24×12	20—4500/ml.	1·03—23·7	
	Cryptomonas sp.	22×8	160—16,000/ml.	8·7—22·6	
	Prorocentrum micans	43×27	5—540/ml.	5·3—42·5	
	Prorocentrum triestimum	10×14	102—1220/ml.	3·5—13·7	
	Gymnodinium vitiligo	9—14	78—7880/ml.	6·2—13·6	

agitation was different for each vessel because of the size of the vessel. Reproductive rates were estimated from a parallel experiment with culture, but no animals. The results are set out in Table 2. There are the following conclusions to be drawn:—

(i) the value of "volume swept clear" was dependent on the size of vessel used;

(ii) the quantity grazed, Q, in the larger vessels is much in excess of the animal's needs (if *S. costatum* is 200 μ^3, Q in the 500 ml. experiment is about 0·9 mg. carbon; the carbon content of *Temora longicornis* is measured in μg.).

It is clear from this experiment that estimates of "volumes swept clear" from experiments made so far are inadequate for population work.

It is possible that during spring outburst in temperate seas the grazing activity of the herbivores is indeed in excess of their needs as Harvey, Cooper, Lebour, and Russell (1935) suggest. Riley, Stommel, and Bumpus (1949) describe experiments which show that this can take place. In December 1947 experiments were made with samples of "phytoplankton" and "zooplankton" taken straight for the nets. Consumption was reckoned as plant pigment units (PPU)/mg. wet weight of zooplankton and the ratio of *carbon consumed* to *carbon required* was unity at 13 PPU/litre, which was four to ten times the surface concentration. At 35 PPU/litre, the ratio increased to 6; this is a little less than the level of the spring outburst on Georges Bank (45 PPU/litre); so it appears that the animals might starve in winter and might feed to excess during the spring increase. Marshall and Orr (1956) have recently repeated their oxygen experiments at different temperatures; during winter the maintenance ration is less than previously thought, but during the spring outburst it is much greater. Thus although the animals may just escape starvation in winter, they must feed heavily in spring.

Plankton Populations

Harvey *et al.* (1935) showed that the production of algae estimated from the decrease of phosphorus was greater than the counted quantity by many times. For example, during the spring outburst in 1934, the phosphorus content of the algae amounted to 0·08 mg. P/1000 PPU and the phosphorus decrease was from 6—8 mg.P/m^3. Hence the total production was

$$\frac{6 \text{ (or } 8) \times 1000}{0{\cdot}08} = 75\text{—}100{,}000 \text{ PPU/m}^3.$$

Table 2. A grazing experiment carried out in vessels of different volumes

Reproductive rate, $R = \log_e \cdot \frac{P_1}{P_0} = \log_e \cdot \frac{164{,}694}{158{,}851} = 0{\cdot}0354$

Volume of vessel	5 ml.	25 ml.	100 ml.	500 ml.
P_0, numbers/ml.	158,851	158,851	158,851	158,851
P_1, numbers/ml.	16,017	58,941	96,656	90,335
$\log_e \frac{P_1}{P_0} = R - G$	2·23	0·99	0·49	0·56
G, daily grazing rate	2·2654	1·0254	0·5254	0·5954
V, volume per animal	2·5	12·5	50	250
$F (= V G t$, where t is 1)	5·66	12·88	26·3	150
$\frac{P_0 \cdot G}{R-G}\left(e^{(R-G)t} - 1\right)$ the quantity grazed, S,	716,644	2,583,743	6,590,156	36,138,463
S/animal/ml.	71,664	51,675	32,951	36,138

The standing stock at any time was never greater than 3000 PPU/m³. However, the standing stock was estimated from fine net catches (silk No. 20) and so was only a fraction of the true value. From a comparison of water bottle samples with fine net catches in the North Sea in spring, it was shown (Cushing, 1955) that a water bottle sample contains 10—15 times as much as the fine net in numbers. As the fine net catches larger diatoms, this is a maximum ratio when considering PPU, which is proportional to volume. Thus, it is likely that the true standing stock was of the order of 20—50,000 PPU/m³.

The difference between the two quantities, production and standing stock, is the quantity eaten or sunk. No diatoms were found on the bottom, for samples of water were taken with a pump within a few inches of the bottom, which was clean and gravelly, "well scoured by tidal streaming". But a clear relation was shown between the standing stock of algae and the number of faecal pellets per animal in the sea. Thus, production was consumed by grazing and not reduced by sinking or "natural" mortality.

An outcome of this work was the mathematical treatment of the problem by Fleming (1939). With the use of arbitrary parameters, curves of algal production were constructed which showed that the typical "bell-shaped" curve of the algal standing stock during the spring outburst could be described by an equation, postulating only a reproductive rate and a grazing rate.

Riley (1946) has used a more complex form of the same equation,

$$\frac{dP}{dt} = \left\{\frac{pI_0}{kz_1}(1 - e^{-kz_1})(1 - N)(1 - V) - R_T - gZ\right\} P,$$

where P is grammes phytoplankton carbon/m²., estimated from chlorophyll measurements,
I_0 is radiant energy g. cal./cm²./min. at 0 m.,
k is the extinction coefficient of visible light.

(Note: the energy extinction coefficient is not strictly linearly related to the depth.)
z_1 is the limiting depth of the euphotic zone at 0·0015 g.cal./cm²./min.,
p is a constant in $Ph = pI$, where Ph is photosynthesis and I is radiation,
$(1 - N)$ is a correction factor for nutrient depletion,
$(1 - V)$ is a correction factor for vertical turbulence,
R_T is the algal respiratory rate,
$gZ = G$, the grazing rate.
Z is the quantity of zooplankton in gC/m². and g is a constant expressing the minimal need for food, based on 1·3—7·6 % of their carbon content.

Riley made five observations during the year of nutrients, turbulence and plankton, and combined them with more numerous observations of radiation and extinction; the equation was calculated for fortnightly intervals to describe a curve which fitted the observations quite well.

The fundamental equation may be expressed as $\frac{dP}{dt} = P(R - G)$ where R is the algal reproductive rate.

The algal populations are then described as being balanced between reproductive rate and grazing rate. With a more complex equation, the greater the number of factors, the greater the possibility of a change in numbers being attributed to the wrong cause. For example, let us suppose that photosynthesis is not linearly proportional to the phosphorus content of the sea. Table 3 shows part of Riley's (1946) calculation recomputed on this supposition.

If the factor $(1 - N)$ is excluded, a two-fold difference appears. Hence, in Riley's equation, the effect of reduced nutrients is decisive in reducing the numbers of phytoplankton; although G, the grazing rate, increases sharply at the right time, its effect is not sufficient. If one parameter in the equation is doubted then a second countering one must also be doubted. For example, if the factor $(1 - N)$ were abandoned, Ph would have to be reduced by other means, perhaps by increased grazing. That this is possible is indicated by the experimental evidence that animals graze in excess of their immediate needs; Riley's grazing coefficient is based on their minimal needs.

Steele (1956) has used a method deriving from Riley's work, based on the phosphorus uptake of the algae. A change in the phosphorus concentration

Table 3. RILEY'S 1946 data recomputed without the factor (1—*N*)

Fortnight	1—*N*	*Ph*	*RT*	*G*	*P*/*dt*	P_1 (after RILEY)	P_2 (without use of 1—*N*)
1	1·00	0·091	0·021	0·043	0·027	10·0	10·0
2	1·00	0·120	0·021	0·056	0·043	19·1	19·1
3	1·00	0·130	0·021	0·067	0·042	35·5	35·5
4	0·95	0·131	0·024	0·129	—0·022	25·7	28·4
5	0·92	0·132	0·026	0·145	—0·039	14·2	18·7
6	0·90	0·134	0·030	0·141	—0·037	8·2	13·4
7	0·88	0·134	0·035	0·105	—0·006	7·5	16·1

Note: $Ph = \frac{pI_0}{kz_1}(1-e^{-kz_1})(1-N)(1-V)$.

from time t_0 to time t_1 at the same position is due to mixing and to "biological change". The two effects were separated in the following way:—

$$\int_{140}^{z}(k-c)\,dz = \int_{140}^{z}(p_2-p_1)\,dz - \frac{p^1z}{T^1z}\int_{140}^{z}(T_2-T_1)\,dz,$$

where z is depth in m.,

K is the regeneration rate of phosphorus, p, to the inorganic state,
C is the consumption rate of p by the algae,
T_1 and T_2 are temperatures at time t_1 and t_2,
p_1 and p_2 are phosphorus contents at time t_1 and t_2,
$p^1z = p_{z+10} - p_{z-10}$,
$Tz^1 = T_{z+10} - T_{z-10}$.

Production is then estimated using a fixed carbon/phosphorus ratio, from the decrease in phosphorus.

Considering reproduction, turbulence, sinking and grazing,

$$\frac{\delta P}{\delta t} = (Ph - R_T - gZ)\,P + \frac{\delta}{\delta z}\left(\frac{A}{\varrho}\frac{P}{z}\right) - V\frac{\delta P}{\delta z},$$

following RILEY's formulation, where

A is the coefficient of vertical diffusivity,
V is the sinking rate,
ϱ is density.

This may be integrated approximately:—

$$P_r = \int_{140}^{z}\left[P_2 - P_1 + \frac{1}{2}G\Delta t\,(Z_1P_1 + Z_2P_2)\right]dz - \frac{\delta P/\delta z}{\delta T/\delta z}\int_{140}^{z}(T_2-T_1)\,dz - \frac{1}{2}\Delta\, tv\left[P_1+P_2\right]_{140}^{z}$$

where P_r is production below Zm in time interval Δt,
P_1 and P_2 is the algal population at t_1 and t_0 ($t_1 - t_0 = \Delta t$) measured as chlorophyll,
$\frac{\delta P}{\delta z}$, $\frac{\delta T}{\delta z}$ are mean gradients of chlorophyll and temperature at depth z,
Z_1 and Z_2 are herbivore numbers at t_1 and t_0.

Using simultaneous equations corresponding to the depth intervals 0—z_0 and z_0—140 m. where z_0 is the depth of zero change in P, solutions for G and V are obtained. The filtering rates varied from 1·0—2·9 litres/mg. C of zooplankton/day. In this formulation an underestimate of G results in an overestimate of V and *vice versa*. During the period of the spring increase, STEELE's solutions gave $V = 13{\cdot}0$ m./day, which seems rather high and suggests part of this may be composed of a grazing effect.

CUSHING (1955) tried to estimate G by first estimating R, i. e.,

$$G = R - \frac{1}{t}\cdot\log_e\frac{P_1}{P_0}$$

The division rates were estimated from the decrease in cell width of some diatoms, over a period during which it is likely that the same populations were being sampled—although doubts could be raised on this point; the division rates varied between 0·1 and 2·9 with an unweighted mean of 0·40. From these estimates it was calculated that *Calanus* was sweeping clear more than 1/litre/day.

There is a further difference between my results and those of RILEY and STEELE; the algal populations were estimated as algal volume from counted numbers raised by the average volume of each species. RILEY and STEELE estimated the algal populations by means of chlorophyll. Both methods have disadvantages. In the algal volume method, it is assumed that all the volume is composed of organic material; HARVEY (1950) has suggested that it is partly composed of cell sap, in which the organic content is presumably reduced. In the chlorophyll method, it is assumed that all chlorophyll measured is living, which is probably not so (GILLBRICHT, 1952) particularly after the spring outburst has been reduced by grazing. However, both methods have one point in common—they overestimate the quantity of living organic material in the sea. The overestimate in algal volume is probably constant, whereas that in chlorophyll probably varies with grazing activity. The first may be used to give unbiased estimates of R and G, but it is possible that the estimates used from the chlorophyll method are biased, because the degree of reduction after the spring increase is not fast enough or

great enough. It is after the spring increase that the dead chlorophyll is likely to appear.

Here I should mention that during the summer, μ-flagellates are also likely to appear, which cannot be counted by the sedimentation techniques. They are certainly of importance in Danish coastal waters as Steemann Nielsen has shown. They will contribute to chlorophyll observations and will slip through the filtering setae of *Calanus* and *Acartia*. There are alternative possibilities:—

(i) in summer, copepods in coastal waters live on μ-flagellates which are numerous enough to feed them by blind filtration,

(ii) in summer copepods do not live on μ-flagellates, but the latter are estimated in chlorophyll determinations, so they appear to do so.

Summarizing the rather meagre results of work on plankton populations, there have been three attempts since the work of Harvey *et al.* to estimate the factors controlling the algal outbursts, each leading to differing results. The effect of grazing in reducing the algal population has not been separated decisively from other factors; the population work so far has only shown that it is possible to set up realistic models.

Discussion

Two points emerge:—

1. The results of experimental work on grazing are not yet sufficiently extensive to be used for the construction of a theoretical grazing rate in the study of plankton populations.
2. There are large differences in the estimates of grazing rate arrived at in the population estimates made so far.

To my mind, the essential point is that the herbivores may feed to excess during the spring increase, as originally suggested by Harvey *et al.* (1935); this finds some support in some experiments described by Riley *et al.* (1949), and that reported here by Cushing and Nicholson. The excess food is excreted and is used during the summer by other parts of the food chain.

The same point can be made in this way:—

For *Calanus* V.

Displacement weight	Dry weight	Daily need of dry weight	Daily need of displ. weight
	($^1/_9$ of displ. weight. Deevey, 1952)	(5% of dry weight of Marshall and Orr, 1935).	(dry wt. $\times$ 9)
0·59 mg. .... (Bogorov, 1934)	0·065 $\times$ $^1/_9$	0·0033	0·03 mg.

The peak algal volume of the north-east coast of England in 1949 was 0·5 mm^3./l. So one *Calanus* V must sweep clear

$$\frac{0{\cdot}03}{0{\cdot}5} \times 1000 \text{ ml./day} = 60 \text{ ml./day}$$

in order to survive. Before and after this time, the volume must have been much higher. Therefore if the herbivore is going to graze to excess, up to six times its daily need, the volume swept clear must indeed be raised, perhaps to as much as 1 litre/day.

To review the effect of grazing on primary production I have touched on nearly every facet of production. This is because I believe with Dr. Harvey (1935) that algal production is usually controlled by grazing: my reasons for believing this are that as herbivore egg production is linked to the quantity of available food, the herbivore production is linked to the algal production. Yet it is an observed fact that as the algae decrease, so the herbivores increase; the nutrients usually start to decline at the point in time at which the algae reach their peak. Nutrients tend to decline as herbivores increase, rather than as algae decline. Hence, it is reasonable to say that the algae are reduced by the herbivores.

To check this we need to know the grazing rates of the herbivores. It is likely that to estimate G from experimental work requires an extensive labour. Perhaps it could be estimated from a simplified form of population equation, e. g.,

$$\frac{dP}{dt} = P(R - G),$$

knowing R.

Thus to determine the true part played by grazing in the sea, it is suggested that our aim should be to arrive at clear and unequivocal measures of the algal reproductive rate.

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D. 4.

External Metabolites and Productivity

By C. E. Lucas

It is nearly 50 years now since Pütter set out his views on the nutritive value of dissolved organic compounds in the sea, and since Allen announced his first observations on the need for diatoms in artificial culture of minute amounts of "natural" sea water, etc. But most of the slight but striking progress since then has been made fairly recently. On the one hand, improved methods have permitted more reliable estimates of the *quantities* of dissolved and colloidal organic matter in natural waters, and on the other observation and experiment has begun to illustrate something more definite of the potent *qualities* of some of these—mainly those which are least abundant and often acting in concentrations of the order of 1 p.p.m. or much less.

I should first draw your attention to the review by Vallentyne (1957) of the extensive literature on the occurrence of organic matter in lakes and sea waters, and incidentally in sewage and soils. Very properly he comments on the comparative unreliability of many early observations, which have depended on the results of destructive analyses, leading to the identification of substances which most likely were not present in the original media (he also makes a plea for "more fruitful co-operation between limnologists and soil scientists"). There seems little doubt that some of the more abundant organics will prove to have straightforward nutritive significance for many plankton heterotrophs and phagotrophs and, despite Krogh's (1931) classic disposal of Pütter's theory, it is not impossible that some macro-molecular organics may yet prove to have crude food value for higher animals (see, for example, Morris (1956) on young fish). In yet another field dissolved or colloidal matter has been observed to influence the pumping rate and therefore presumably the feeding rate of oysters (Collier, Ray, Magnitski, and Bell, 1953). In addition, there is some evidence that free organic substances may influence higher fish in their migration (e. g., Hasler, 1954), just as we know how "smells" may on a more local scale influence animals in their search for food or for their mates.

To-day, however, we are concerned with productivity and particularly with substances dissolved in extremely low concentrations. Obviously, we are only at the beginning of this type of investigation, but recent work is showing clearly how important were Allen's early observations and those of the very few subsequent workers, such as Gran and Akehurst, who saw successful growth in terms of "succession" and the non-predatory significance of one organism for another in the community.

This can only be a brief review of some recent progress in a relatively new subject, since reference can also be made to previous reviews (Lucas, 1938, 1947, 1949, and 1955), in which this aspect of the significance of external metabolites has been discussed along with others. A tentative thesis was made there, with references, concerning (a) the tendency for organisms to release metabolites into the external medium, including physiologically potent metabolites (ectocrines), (b) for "successful" organisms to adapt themselves to the existence of such metabolites in their environment, so that among other things, (c) some elements of the community might appear to be benefited (in the "vitamin" sense) or harmed (in the "antibiotic" sense), bearing in mind (d) that one organism's food might well be another's poison!

A number of recent references were mentioned in the last review (1955). The work of Droop (1954), and others, Harvey (1955), Johnston (1955), Lefèvre, Jakob and Nisbet (1952), Lewin (1954), Pratt (1943), Provasoli and Pintner (1953) and Rice (1954) will serve as reminders. But I will specifically remind you that they and others between them had demonstrated (a) either favourable or unfavourable interaction between micro-organisms grown together, (b) similar interactions when such organisms were grown in the medium of another, and (c) the extraction of substances having such effects from natural

or culture media. In particular, both LEWIN and DROOP had shown the significance of vitamin B_{12} for some algae while PROVASOLI (and others) had stated in more specific terms than I did the general case for the significance of such reactions in ecology.

More recently published work takes us further. FOGG and WESTLAKE (1955) have revealed the likelihood that the extensive release of polypeptides by blue-green algae may, by formation of complexes, have beneficial effects in ecology. JØRGENSEN (1956, 1957) has given further instances of auto- and heterotoxicity and acceleration among algae, with marked variability relative to culture-age and experimental conditions. PROCTOR (1957) has given yet another instance of interspecific inhibition, with some evidence that it may depend on the release of fatty acids in this case, and has emphasized the great importance of experimental conditions in all such work, particularly the age of the cultures. He considers it uncertain whether "inhibitors" are ever produced by healthy cells, while admitting that in ecology this may matter little since healthy cells and damaged ones are both abundant. Further, DROOP (1957) has made progress in defining culture media and growth conditions for selected micro-algae, including pelagic ones, while COWEY (1956) demonstrated with samples collected by our vessels the existence of an annual cycle of B_{12} in the sea, with a winter maximum of 2 mμg./l. and a summer minimum of 0·1 mμg./l. Again, SWEENEY (1954) has shown that *Gymnodinium splendens* can only be cultured in the bacteria-free state if B_{12} is available (optimum concentration 0·01—0·1 μg./l.).

The recent work on B_{12} points the way towards more specific understanding, although it will probably not prove to be an easy way. Already there is not only the problem of B_{12} analogues to be faced, but also that of "bound" B_{12} with the associated uncertainty regarding the significance of total B_{12} as distinct from "nutritive" B_{12}. Doubtless B_{12} will prove to be only one of many potent metabolites or "ectocrines" since the needs of pelagic auxotrophs are likely to be many and various. Another and very different group of substances is being investigated by Dr. JOYCE BENTLEY (Senior Research Fellow at Aberdeen). Following her work elsewhere on the hormones of higher plants, she has been studying the occurrence of the more familiar ones, and others, in the pelagic algae and in natural waters. An account of the first results will be published soon and I am grateful to her for permission to quote the following in advance (see also LUCAS, 1957):—

"It was thought that it might be profitable to investigate the effect of plant hormones on the growth of phytoplankton, and whether they produce these hormones and secrete them to external medium. Plant hormones have only been studied critically in the higher plants, yet they affect fundamental aspects of growth, e. g., cell elongation and cell division. Their primary effect is on the individual cell, and as such is likely to be of importance in the growth of phytoplankton.

Work has been in progress along these lines in this laboratory for the last two years and, although only preliminary as yet, has yielded some interesting results. Firstly, there is clearly an effect of applied 3-indolylacetic acid (IAA—one of the naturally-occurring plant hormones) on the growth of a number of marine phytoplankton species in culture. This was particularly marked with *Skeletonema costatum* maintained in bacteria-free culture (kindly supplied originally by Dr. DROOP) in a synthetic nutrient medium. Under these conditions IAA causes an increase in cell number and chain-length at concentrations of 10^{-10} to 10^{-12} g./cc. Secondly, extracts of various algal species have been analysed, and results have shown that these organisms produce a range of plant hormones. Some are probably identical with the known plant hormones, but some are as yet chemically unidentified. Lastly, it has been established that some of these hormones exist free in sea water and in fresh water, from which algal material was collected. Thus it is clear that these substances must be taken into consideration in theories on the ecological effects of traces of organic substances in natural waters. Work is continuing on the distribution and identification of these hormones".

This then is another and promising line of investigation and doubtless yet more will be found. But how important are these possibilities to us? They suggest that the understanding of production requires knowledge of more than inorganic nutrients.

Inhibitors, when present, are limiting enough; we must be prepared, however, for some organisms to benefit from the inhibition of others. As regards "accelerators", at first sight we are concerned with substances which are either valuable or are "absolute" growth accessories, however abundant may be the supplies of nutrients, and however suitable the physical conditions. They are controlling factors (subject to nutrients) and DROOP (1954) is already able to say of B_{12} for example that "the requirement, when present, is absolute and demonstrable by a clear dose/response relation". Many of the beneficial or harmful substances which will in due course appear to be significant are doubtless concerned in the striking but hitherto baffling process of "succession", the existence of which was itself one of the early reasons for doubting the complete adequacy of nutrients as ordinarily understood. To be successful, or a "successor", an organism must have adapted itself to the results of the activities of its predecessors in the community (as is typical also of other aspects of life). Chemically speaking, these activities seem to be far more extensive and potent than was appreciated until recently, and even when considering the growth of "pure" species, this may be important (e. g., from lag-phase to rapid growth and then senescence). These considerations alone suggest the value of continuing and extending the investigation, quite apart from the possible commercial value of metabolites which may yet be extracted in economic quantities from either the plants themselves or their media.

In another paper in this Symposium, however, Mr. STEELE has discussed the different methods and results of estimating production and has, with the aid of a mathematical model, given some insight into the complex factors controlling production in the northern North Sea. But, as he says, one disadvantage common to such methods is that the approach "has tended away from the traditional biological units and deals instead with such abstractions as "carbon content" ..... to achieve a generality which allows comparison ..... In particular, it seems impossible to discover any link between quantitative studies of production and the data from the rapidly developing study of the effects of "metabolites" .....". It may be paradoxical, but apparently it is the case that a considerable understanding of production can be obtained in this way, without any consideration (except perhaps in general terms) of the specific composition of at least phytoplankton; and we have to recognize that the results obtained in this field by workers such as RILEY, HARVEY and STEELE have been very useful.

What is the solution? It is early to say, but it may prove to be true that production potential in terms of phosphate, nitrate, etc. and subject to physical conditions such as vertical mixing, almost or entirely determines the bulk of phytoplankton and zooplankton, and even of fish. But the specific nature of the community, which is so interesting and important in other respects, may still be important in this. Not only are some species more interesting and signiffcant at both the scientific and the aesthetic levels than others but, even at the crude level of food value, both on land and in the sea, some are more valuable than others to those in the community that have become adapted to them for one reason or another. Not only may this be the case in general, but there are an increasing number of examples where the success or failure of an animal is known to be determined by the specific nature of the food available at a critical stage (for example, the larval stage in oyster culture). Recent pointers towards the significance of the "right" conditions for the successful development of eggs and larvae of fish suggest that these may also depend on the specific nature of, for example, the animal plankton available at a critical time (e. g., *Oikopleura* for the young plaice; SHELBOURNE, 1957).

We cannot yet be sure but, if this approach is along the right lines, we may be able to say that basic physical and inorganic chemical conditions tend to determine the volume of the ecological pyramid of numbers in a given situation, and that this volume will be occupied by appropriate proportions of plants and animals, but that, from the human point of view—whether aesthetic, scientific, or nutritional—the important question will still be "with what kinds of plants and animals is it occupied?" Are they "right" kinds, either to provide directly the food for the next link of interest to us in the food chain or, indirectly by metabolite release, to provide conditions suitable for that or any other link which is obliged to depend on the metabolism of others for its own success?

This makes the story more complex, but it provides very strong reasons for considering not only the composition of the phytoplankton community, but also its various metabolites, in relation to productivity in the sea—while still being grateful for the more generalized picture provided by the abstract quantitative approach.

In conclusion, I might mention a point made by my colleague Dr. JOHNSTON. It is perhaps inevitable that, in this preliminary work, clues should have been sought within our still limited knowledge of the metabolism of higher plants and animals, e. g., auxins and vitamins—and not without success. But this approach may yet prove a limited one and new approaches may be revealed as we begin to understand better the chemistry of the lower forms and their metabolism.

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